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VARIABILITY AND AMPHIMIXIS

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A COMPARATIVE STUDY OF THE VARIABILITY IN ZYGOSPORES OF *Spirogyra inflata* (VAUCH.) FORMED BY LATERAL (CLOSE BREEDING) AND BY SCALARIFORM (CROSS BREEDING) CONJUGATION, AND ITS BEARING ON THE THEORY OF AMPHIMIXIS AND CORRELATED PROBLEMS

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I. PRELIMINARY OUTLINE

1. Introduction

COMPARATIVE studies along statistical lines of the results produced by cross breeding and close breeding afford data of value bearing on the problem of evolution as well as the subsidiary problem of the origin of amphimixis. It has long been assumed (Weismann, '76) that sex existed primarily to increase variability and with the further assumption that the variations thus produced were heritable and accumulated, the differentiation of organisms was logically explained. As a corollary to such a conclusion the belief has long been prevalent that the offspring of organisms produced by cross breeding were as a group more variable than those produced by close breeding, an idea which gained further acceptance in connection with the investigations of Castle ('06), Jennings ('08, '09, '12, '13) and others interested in problems of genetics. That there was excellent evidence for exactly an opposite view and that an analysis of the results presented by the investigators mentioned above did not bear out the conclusion that variability was increased by cross breeding has been pointed out by the writer (Walton, '08, '12, '14) in some earlier papers.

The importance of arriving at a correct conclusion concerning the part played by hybridization and cross breeding in evolution can not be overestimated. If units are merely redistributed and form characters resulting in no actual evolutionary progress, work along Mendelian lines tends rather to obscure the facts of value toward solving the problem of the origin of species as well as that of evolutionary control in animal and plant breeding. It is therefore well to obtain data from as many sources as possible bearing on the question.

Among the species of *Spirogyra*, a group of algæ belonging to the class Conjugatæ, there are several which reproduce both by lateral conjugation (Fig. 1, A) where

the adjacent cells of a single filament unite to form the zygospore, itself a young individual, and at the same time by scalariform conjugation (Fig. 1, *B*) where the cells of two distinct filaments unite to form the zygospore. Thus

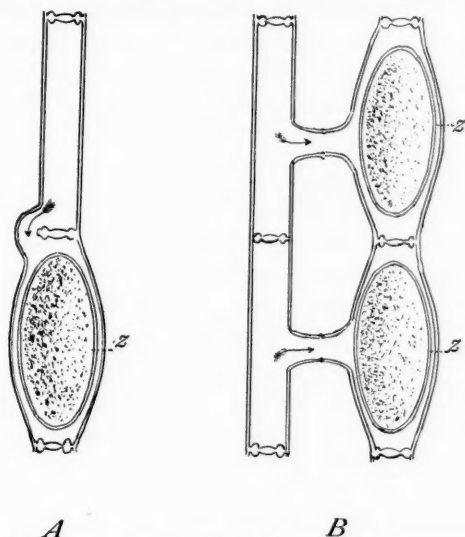


FIG. 1. FORMATION OF ZYGOSPORES IN *Spirogyra inflata* (Vauch.) by lateral conjugation (*A*) close bred from the same filament, and by scalariform conjugation (*B*) cross bred from two distinct filaments. *z* = zygospore.

there is an example of a population producing under the same environment two groups of individuals, one by close breeding (lateral conjugation) and the other by cross breeding (scalariform conjugation), and a comparison of the variability by statistical methods should afford evidence toward the solution of the problem presented where the offspring have arisen from a common ancestor as indicated in the material studied.

2. Historical

Much has been published concerning hybridization, cross and close breeding, amphimixis and parthenogenesis, all of which are distinguishable from one another merely by degree, nevertheless so far as the subject under

discussion is concerned, the conclusions in general have largely been assumptions based on little or no evidence.

It was Weismann ('76) who was evidently the first to definitely express the importance of sex in producing variations, an idea to which he consistently held in his subsequent papers, while Nägeli ('84), Strasburger ('84), Hatscheck ('87), Huxley ('95), etc., believed likewise on theoretical grounds that variability was reduced by amphimixis.

The first paper presenting tangible evidence upon the subject was that of Warren ('99) who found that parthenogenetically produced *Daphnia magna* were slightly more variable as measured by the "Standard Deviation" which had a value of 2.95, than the mothers whose "Standard Deviation" was 2.22. The small number utilized, 96 in the first instance and 23 in the second instance, together with the fact that the mothers represented a selected class, only those *Daphnia* producing young being included, did not allow placing much reliance in the results. Warren ('02) compared 60 parental aphids (*Hyalopteris trirhodus*) and their 368 offspring as well as a series from 30 aphid grandparents and their 291 grandchildren. The variability was found in a comparison of grandparents and grandchildren (parthenogenetic) to have slightly decreased in respect to frontal breadth and considerably increased in respect to length of right antenna, but again objections similar to those in the preceding paper render the conclusion of little value, as Warren himself observed.

Casteel and Phillips ('03) measured drones and workers of *Apis mellifica*, the honey bee, selecting individuals at random from different colonies, and tabulating classes and frequencies without, however, a further application of biometrical methods. The "range of variability" was found to be greater in the drones than in the workers. Lutz ('04) criticized the methods utilized in the paper, nevertheless variation as measured by the standard devia-

tion upon calculation by Wright, Lee and Pearson ('07) was found greater in the drones by a difference ranging from 0.22 to 2.63 in respect to all five characters studied in the single group of 50 Italian workers and 50 drones of real value for comparative purposes.

Kellogg ('06), in a preliminary paper dealing with drones and workers of bees and also with female aphids, concluded that not only was there no evidence that amphimixis produced increased variability, but that it was an unnecessary factor in the production of Darwinian variation. The results were summarized as follows:

(a) In all but one of the characteristics studied, the amount of variation both quantitative and qualitative, is markedly larger among the drone bees than among the workers, and in the one exceptional characteristic it is no less; (b) no more variation in wing characters is apparent among drones or workers that have not been exposed in imaginal condition to the rigors of personal selection than exists among bees, drones or workers, that have been so exposed; (c) the variation in wing characters in drone bees reared in worker cells is no greater than that among individuals reared among drone cells; (d) the variation among drones hatched from worker laid eggs is markedly larger than that among drones hatched from queen laid eggs. . . ."

Eleven "lots" were studied with a small number (No. 3, 48; No. 7, 54; No. 8, 75; No. 9, 26; No. 11, 60) in many of the "lots." Even though the probable errors would have been large and while the material was heterogeneous, the facts brought out are of extreme interest, particularly when considered with the results obtained by Casteel and Phillips ('03).

Wright, Lee and Pearson ('07) made a comparative biometrical study of 129 queens, 130 drones, and 129 workers taken from a nest of the common wasp *Vespa vulgaris* in Charterhouse, England. In connection with the wing dimensions, the coefficient of variation was found to be greatest in the worker, less in the drone, and least in the queen, differing from the bee as noted above where drones were more variable than workers. The conclusion here of interest was:

There is no evidence in favor of parthenogenesis resulting in a smaller variability than sexual reproduction, for if the workers be more, the queens are less, variable than the drones.

It was suggested by the writers that the large variabilities of the workers might have resulted from subclasses among them due to differentiated functions or natures.

Castle, Carpenter, Clark, Mast and Barrows ('06) made observations on the variability and fertility of *Drosophila ampelophila* Loew, the small fruit fly, as modified by inbreeding and cross breeding. They found that "inbreeding does not affect the variability in number of teeth on the sex comb of the male, nor the variability in size." While the conclusion is not in accord with an earlier observation (p. 780) that variability would seem to have been increased by inbreeding so far as a comparison of the sixth inbred generation with the sixty-first generation, the small number utilized in the sixth generation (40 males in series A-6, B-6, C-6 each) was ground for the opinion that such a conclusion had little value in comparison with data pointing in the reverse direction. If however we calculate the coefficient of variation for the length of the tibia, an unfortunate omission on the part of the writers, it may be noted that the flies produced by inbreeding are decidedly more variable than those produced by cross breeding. Data for this conclusion are given in a subsequent part of the present paper.

Walton ('08) noted that the results of measuring zygospores of *Spirogyra* indicated that the close-bred individuals were more variable than the cross-bred individuals and furthermore that the data went far toward confirming the theory that sex existed for the purpose of limiting instead of augmenting variability.

Emerson ('10) found that crosses between races of plants (maize, squash, beans, gourds) differing in size and shape had the variability of the second (F_2) generation approximately twice as great as the variability of either parental form or of the first (F_1) generation. This he

explained on the basis of the segregation of size and shape characters. Similar results were obtained by East ('11) for maize and Hayes ('12) for tobacco.

Jennings ('11) extending and summarizing his breeding experiments on *Paramecium* concluded that

The progeny of conjugants are more variable, in size and in certain other respects, than the progeny of the equivalent non-conjugants. Thus conjugation increases variation.

Later ('13) continuing his investigations he stated that conjugation increased the variability in the rate of reproduction. In a subsequent part of the present paper a somewhat critical review of the data and conclusions therein noted is presented.

3. Material

In obtaining material early one April for the laboratory work of a class in biology, the collection being made in a small pool resulting from the overflow of a rivulet, a peculiar species of *Spirogyra* was noticed in which both lateral and scalariform conjugation was taking place often in the same filament. It was at once suggestive that a comparison of the variability in the two groups of zygospores would present facts of interest in connection with the effect of close breeding and cross breeding on variability as well as affording evidence as to the theories of amphimixis.

The species was first determined as *Spirogyra quadrata* (Hass.) but subsequent examination indicated that it should be classified as *Spirogyra inflata* (Vauch.).

The material utilized for the measurements was all procured at one time from a restricted area one or two inches square on the surface of the pool and included only the one form of *Spirogyra*, that alone being present as a mass 3 or 4 inches in diameter. Inasmuch as both lateral and scalariform conjugation occasionally took place in the same filament (Fig. 2) a suggestion that two species were

represented can not be made for the filaments are alike in every characteristic. Of the 500 zygospores observed 45 per cent. were produced by lateral conjugation.

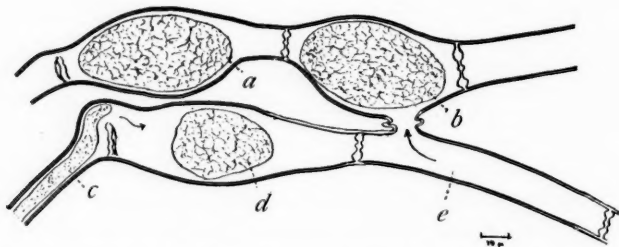


FIG. 2. *Spirogyra inflata* (Vauch.) $\times 800$, with both scalariform and lateral conjugation in the same filament. (a) Zygospore formed by lateral conjugation. (b) Zygospore formed by scalariform conjugation. (c) Protoplasm of a "male" cell uniting with (d) the protoplasm of a "female" cell to form a zygospore by lateral conjugation. (e) Cell from which the protoplasm has passed to form the zygospore in (b). Obj. 1/12, Ocul. 2. Camera lucida drawing.

4. Methods

In considering the variability of large numbers of microscopic forms, rapid and accurate measurements are a necessity. Pearl and Dunbar ('03) in measuring *Arceella* used a camera lucida, marking the dimensions by means of a needle point, and reducing to microns. Pearl ('06) adopted a similar method for *Chilomonas*, using a magnification of 689.7. Pearl ('07) in measuring *Paramecium* used a 2/3-inch objective and a No. 1 ocular. By means of a camera lucida the points to be measured were projected on cards, marked, and measured with a vernier calipers to tenths of millimeters. Multiplying the measurements so obtained by the proper reduction factor found by calibrating with a stage micrometer, they were reduced to microns and recorded. Jennings ('11) at first measured *Paramecium* from a slide with an ocular micrometer. Later an Edinger drawing and projection apparatus was used, the projected images of the specimens on a slide in a flat drop of 25 per cent. glycerine, with-

out a cover glass which by pressure would have caused distortion, were enlarged to 500 diameters and measured with a millimeter ruler.

In the present study, the material was preserved in 2 per cent. formalin, the first series of measurements¹ being made April 2, while measurements of 358 were completed before May 16, and the remaining 42 finished Aug. 21 of the same year. Swelling of the zygospores did not occur to an appreciable extent, a possible error considered in a subsequent part of the paper. Using a B. and L. BB-6 microscope with a No. 1 ocular and a 1/12 oil immersion, a slide with a couple of drops of fluid containing the material was covered with a No. 2 coverglass, the superfluous liquid drawn off by means of a pipette, and the preparation placed on the mechanical stage. Beginning at the lower right-hand corner the slide was moved from left to right and each zygospore presented in the field in a uniformly horizontal condition, was measured. On reaching the left margin of the preparation, the slide was returned to the first position, moved sufficiently toward the observer so that a new path would be traversed, and the operation repeated. Thus the selection was at random and no zygospore measured twice. The dimensions were marked on note paper by means of a camera lucida at a magnification of 1,460 diameters, the two cross lines representing length (x) and diameter (y) having at the point of juncture an "S" or an "L" for scalariform or lateral conjugation. Only those zygospores having definitely formed membranes were considered.

In the reduction of data, so soon as the projections of the apparent dimensions were completed, the length of the lines x and y were measured with proportional dividers (Keuffel and Esser No. 441 special) adjusted at the ratio 1,460 to 1,000, thus giving a reading in tenths of microns. Accurate adjustment was made possible by means of a

¹ I am indebted to Dr. C. C. W. Judd, of Baltimore, Md., at that time a senior in Kenyon College, for work in part in obtaining the first series of measurements.

micrometer screw, on the basis of the equation for similar triangles;

$$1,460 \text{ mm.} : 1,000 \text{ mm.} = 160 \text{ mm.} - x \text{ mm.} : x \text{ mm.}$$

where 160 represented the total length of the dividers, and x or 65.04 mm. the point of adjustment. Having checked the adjustment, it only became necessary to note the size of a given zygosporc with the longer legs of the instrument, then by applying the shorter legs to a millimeter scale, to read the result. The various constants were then computed on the basis of the work of Pearson and of Elderton by means of a Brunsviga calculating machine. I am indebted to Dr. H. H. Mitchell of the University of Illinois for checking the mathematical data.

II. CONSIDERATION OF RESULTS

The direct results obtained by the statistical methods employed are here presented. These furnish the basis for the general discussion and conclusions which follow. The problems of biology relating to evolution need the application of statistical methods to studies in genetics. In no other way will it be possible to clearly demonstrate the relative efficiency of the different types of variation—fluctuation, amphiutation, cumulation, etc.—in originating and maintaining the diverse forms of life that exist. Similarly the importance or unimportance of small variations in animal and plant breeding may only thus be explained. The refinements of curve fitting are by no means necessary, nevertheless values are thus exhibited which are presentable in no other way.

1. *Comparative Variability in Length of Zygozpozes*

In the frequency distribution for lengths of the two groups of zygozpozes (Table I) the class range adopted was two microns as compared with a range of one micron in the distribution of diameters. The more extended as well as the more irregular distribution of lengths of the lateral zygozpozes when compared with the scalariform

zygospores is at once suggestive that the group thus close bred, is the more variable one. It is also of some interest to note that the empirical range of variation for the laterally formed zygospores,—with length from 49 m. to 83 m.,—is considerably greater than in the scalariform zygospores with lengths from 47 m. to 79 m. While this is not a measure of statistical variability, it undoubtedly has a genetic value.

TABLE I

LENGTH OF 400 ZYGOSPORES FROM *Spirogyra inflata* (VAUCH.), 200 PRODUCED BY LATERAL CONJUGATION AND 200 PRODUCED BY SCALARIFORM CONJUGATION, ARRANGED IN CLASSES ACCORDING TO FREQUENCIES.
MAGNITUDES IN $1/10m$

Length of zygospores in microns.

Lateral Conjugation			Scalariform Conjugation	
Class	Frequency		Frequency	
	Observed	Calculated	Observed	Calculated
38.0-39.9	1	0.21	0	0.
40.0-41.9	0	0.43	0	0.
42.0-43.9	1	0.84	0	0.
44.0-45.9	3	1.49	0	0.
46.0-47.9	2	2.56	3	1.17
48.0-49.9	0	4.18	3	3.14
50.0-51.9	4	6.44	6	6.76
52.0-53.9	16	9.34	16	12.65
54.0-55.9	10	12.71	20	19.82
56.0-57.9	26	16.16	25	26.21
58.0-59.9	11	19.17	25	29.40
60.0-61.9	19	21.15	26	28.41
62.0-63.9	22	21.67	25	24.13
64.0-65.9	20	21.18	18	18.30
66.0-67.9	21	18.10	11	12.66
68.0-69.9	13	14.72	7	8.12
70.0-71.9	16	11.08	6	4.90
72.0-73.9	5	7.72	6	2.83
74.0-75.9	1	4.98	1	1.57
76.0-77.9	5	2.99	1	.85
78.0-79.9	2	1.66	1	.45
80.0-81.9	1	.86	0	0.
82.0-83.9	1	.42	0	0.
Total.....	200	200.06	200	201.04

The general constants for the variability in the length of the zygospore of the two groups are shown below (Table II). It may be noted that the mean (M.) or aver-

TABLE II

GENERAL CONSTANTS FOR VARIATION IN LENGTHS OF ZYGOSPORES BASED ON
200 FORMED BY LATERAL AND 200 FORMED BY SCALARIFORM CONJUGA-
TION WITH A CLASS RANGE OF 2 MICRONS. STANDARD
DEVIATION IN MICRONS

Constant		Lateral Conjugation (Close Bred)		Scalariform Conjugation (Cross Bred)	
Name	Symbol	Value	Prob. Error	Value	Prob. Error
Number.....	N.	200	200
Mean.....	M.	62.38	± 0.1776	60.44	± 0.1345
Mode.....	Mo.	62.5123	59.5170
Standard deviation.....	σ .	7.4460	± 0.1304	5.7474	± 0.1104
Coefficient of variation.....	C.V.	11.9364	± 0.0330	9.5093	± 0.0330
Skewness.....	Sk.	-.0356	± 0.0468	.1589	$\pm .0464$

age length of the zygospores produced by lateral conjugation exceeds the mean of the scalariform conjugants by 1.94 microns, while the probable error for the first constant is $\pm .1776$ and for the second constant $\pm .1345$. The difference is therefore a significant one so far as the present material is concerned.

It is in the comparison of the standard deviations (σ) and the coefficients of variation (C. V.) that the results of most interest appear, however. The former constant in lateral conjugation has a value of 1.6986 in excess of the same constant in scalariform conjugation, or relatively 29 per cent. This is more than thirteen times the probable error. In the coefficient of variation, an abstract number permitting comparison with similar constants in other organisms, the results indicate that the variability in lateral conjugation exceeds that occurring in scalariform conjugation by 2.4271 or relatively 26 per cent., a result corroborated by the distribution of the diameters. The probable errors are sufficiently small in comparison with the differences noted, that they may be considered negligible.

Skewness is negative in the curve for lateral conjugation, the mean being on the left side of the mode, but its value is less than the probable error. In the curve for scalariform conjugation skewness is positive with a value

slightly more than three times the probable error. Therefore the differences of the two constants appear to have no particular value so far as the present material is concerned.

The analytical constants (Table III) necessary for the

TABLE III

ANALYTICAL CONSTANTS FOR VARIATION IN LENGTH OF ZYGOSPORES FORMED BY LATERAL SCALARIFORM CONJUGATION.

Constant	Lateral Conjugation	Scalariform Conjugation
m_2	13.8606	8.2583
m_3	— 3.9024	9.2989
m_4	606.8690	245.0223
B_10057	.1535
B_10756	.3918
B_2	3.1068	3.5928
k0219	.1654

fitting of the curves indicate that type IV curves may be used for each method of conjugation. In lateral conjugation the equation is

$$y = 10.842 \left(1 + \frac{x^2}{29.2687^2} \right)^{-33.0942} \times e^{-9.5995 \tan^{-1}(x/29.2687)}$$

and in scalariform conjugation similarly the equation is

$$y = 5.0014 \left(1 + \frac{x^2}{529} \right)^{-11.6945} \times e^{-8.9889 \tan^{-1}(x/11.56)}$$

while the frequency polygons and the fitted curves (Figs. 3, 4, and 5) illustrate the conditions diagrammatically.

² The following formulæ as the basis of the probable errors, may be noted:

$$PE_{\sigma} = .67449 \sqrt{\frac{M_4 - M_2^2}{4M_2}} / n, \quad PE_{c.v.} = \frac{.67449}{\sqrt{2n}} \left[1 + 2 \left(\frac{v}{100} \right)^2 \right]^{\frac{1}{2}},$$

$$PE_{sk.} = .67449 \sqrt{\frac{3}{2n}} / \sqrt{1 + 3(sk.)^2}.$$

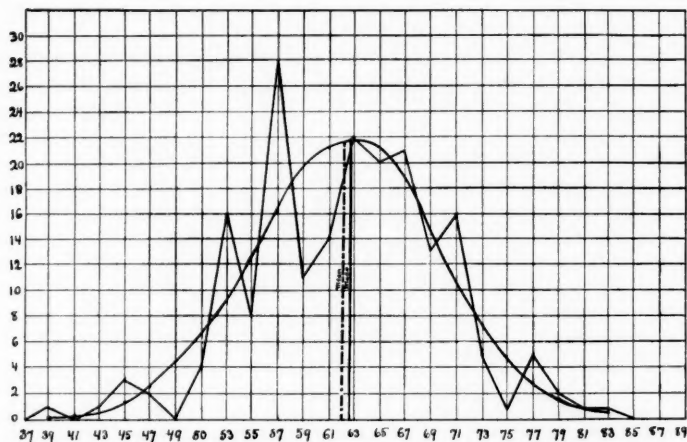


FIG. 3. FREQUENCY POLYGON AND FITTED CURVE FOR VARIATION IN LENGTH OF ZYGOSPORES PRODUCED BY LATERAL CONJUGATION (INBREEDING) IN *Spirogyra inflata* (Vauch.).

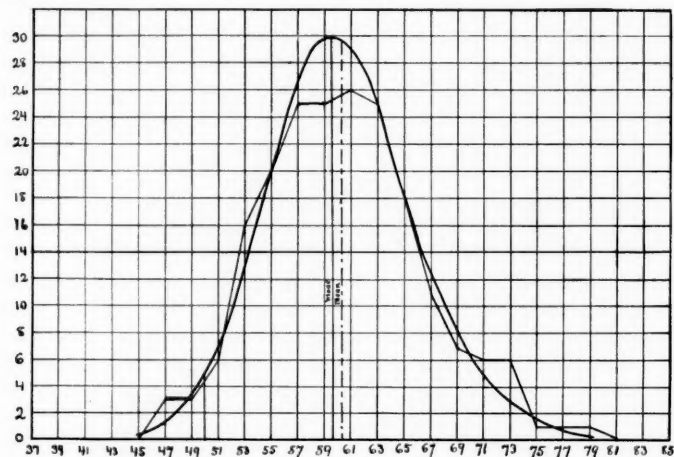


FIG. 4. FREQUENCY POLYGON AND FITTED CURVE FOR VARIATION IN LENGTH OF ZYGOSPORES PRODUCED BY SCALARIFORM CONJUGATION (CROSS BREEDING) IN *Spirogyra inflata* (Vauch.).

2. Comparative Variability in the Diameter of the Zygospores

The class range adopted in the frequency distribution for diameters (Table IV) of the two groups of zygospores

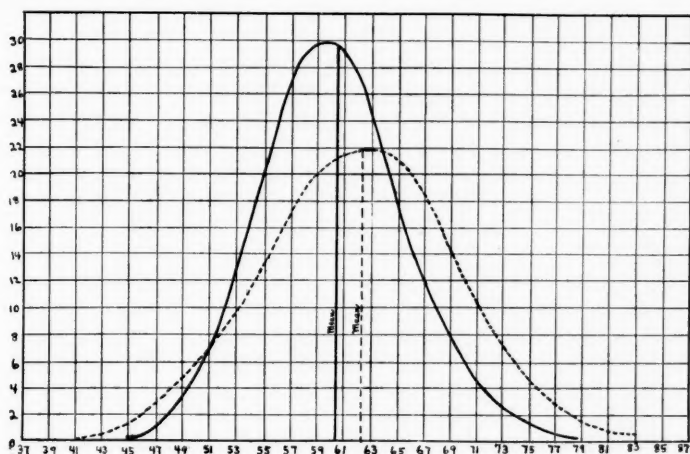


FIG. 5. COMPARISON OF FITTED CURVES FOR VARIATION IN LENGTH OF ZYGOSPORES PRODUCED BY LATERAL CONJUGATION (CLOSE BREEDING) AND BY SCALARIFORM CONJUGATION (CROSS BREEDING) IN *Spirogyra inflata* (Vauch.). Lateral conjugation = ————, Scalariform conjugation = ————.

spores was one micron, measurement being made at the maximum diameter. An inspection of the distribution shows at once the greater concentration of the variates

TABLE IV

DIAMETERS OF 400 ZYGOSPORES FROM *Spirogyra quadrata* (HAAS.) 200 PRODUCED BY LATERAL CONJUGATION AND 200 PRODUCED BY SCALARIFORM CONJUGATION, ARRANGED IN CLASSES ACCORDING TO FREQUENCIES.
MAGNITUDES IN $1/10 \mu$.

DIAMETER OF ZYGOSPORES IN MICRONS.

Lateral Conjugation		Scalariform Conjugation	
Class	Frequency	Class	Frequency
23.0-23.9	0	23.0-23.9	1
24.0-24.9	3	24.0-24.9	0
25.0-25.9	4	25.0-25.9	3
26.0-26.9	12	26.0-26.9	9
27.0-27.9	22	27.0-27.9	13
28.0-28.9	44	28.0-28.9	38
29.0-29.9	40	29.0-29.9	46
30.0-30.9	25	30.0-30.9	46
31.0-31.9	16	31.0-31.9	30
32.0-32.9	15	32.0-32.9	9
33.0-33.9	10	33.0-33.9	4
34.0-34.9	9	34.0-34.9	1
Total	200	Total	200

TABLE V

GENERAL CONSTANTS FOR VARIATION IN DIAMETERS OF ZYGOSPORES BASED
ON 200 FORMED BY LATERAL AND 200 FORMED BY SCALARIFORM
CONJUGATION WITH A CLASS RANGE OF 1 MICRON.

Constant		Lateral Conjugation, Close Breeding		Scalariform Conjugation, Cross Breeding	
Name	Symbol	Value	Prob. Error	Value	Prob. Error
Number.....	N.	200	200
Mean.....	M.	29.66	±.1049	29.725	±.0801
Mode.....	Mo.	29.1577	29.9735
Standard deviation.....	σ .	2.1980	±.0688	1.6796	±.0583
Coefficient of variation.....	C.V.	7.5376	±.0339	5.7471	±.0338
Skewness.....	Sk.	.2285	±.0505	-.1480	±.0566

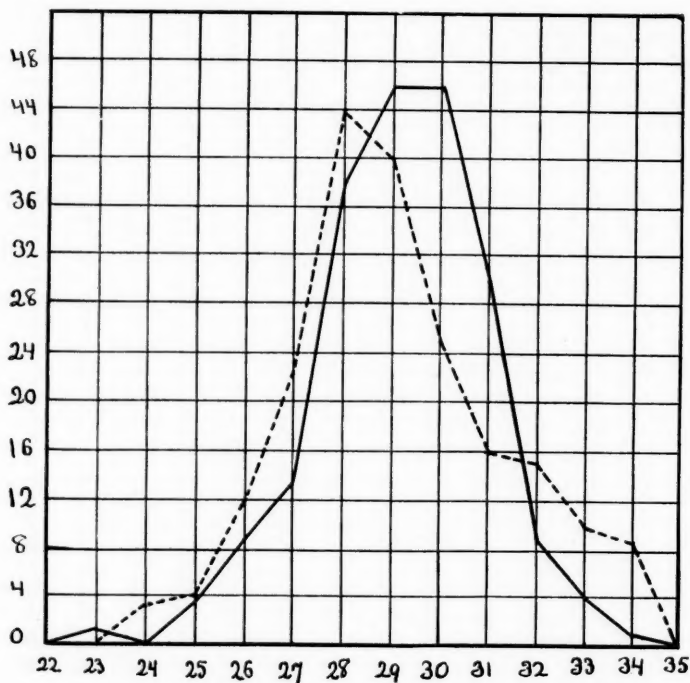


FIG. 6. DIAGRAM SHOWING THE FREQUENCY POLYGON FOR THE VARIATION IN DIAMETER OF ZYGOSPORES IN *Spirogyra inflata* (Vauch.) PRODUCED BY LATERAL CONJUGATION (INBREEDING) AND BY SCALARIFORM CONJUGATION (CROSS BREEDING). The unit of abscissa is one mikron. ----- = Lateral conjugation (close breeding). ————— = Scalariform conjugation (cross breeding).

in scalariform conjugation, and thus their smaller variability. In considering the general constants of variability (Table V) based on the diameters it is to be noted that the means (M.) do not differ, as was found when considering length. The standard deviation (σ) and the coefficient of variation (C. V.) once more demonstrate the greater variability of the laterally formed zygospores. The values of the constants for skewness (Sk.) are not sufficient, however, when considered with the probable error, to be of importance. The frequency polygons (Fig. 6) illustrate conditions, although no curves have been fitted.

3. Comparative Correlation of Length and Diameter

In view of the results obtained in a consideration of the variability, it will be of some interest to ascertain whether

TABLE VI
CORRELATION BETWEEN LENGTH AND DIAMETER OF 200 ZYGOSPORES OF
Spirogyra inflata (VAUCH.) PRODUCED BY LATERAL CONJUGATION

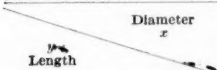
<div style="text-align: center;">Diameter x by Length</div>	23.0-23.9	24.0-24.9	25.0-25.9	26.0-26.9	27.0-27.9	28.0-28.9	29.0-29.9	30.0-30.9	31.0-31.9	32.0-32.9	33.0-33.9	34.0-34.9	Totals
38.0-39.9						1							1
40.0-41.9													0
42.0-43.9									1				1
44.0-45.9			1	1			1						3
46.0-47.9				1						1			2
48.0-49.9													0
50.0-51.9			1		1		1	1					4
52.0-53.9		1		2	3	2	3	2		2	1		16
54.0-55.9				1	2	2	3	2					10
56.0-57.9			1	1	4	6	5	3	2		3	1	26
58.0-59.9						2	4	1	3			1	11
60.0-61.9				1	1	6	6	2		1	2		19
62.0-63.9		1				7	5	4		3		2	22
64.0-65.9		1			2	5	3	1	3	2	2	1	20
66.0-67.9				1	5	7	1	3		2	1	1	21
68.0-69.9					2	3	4	3				1	13
70.0-71.9				4	1	1	3	1	3	1		2	16
72.0-73.9						1	1	1		1	1		5
74.0-75.9									1				1
76.0-77.9			1					1	1	2			5
78.0-79.9					1				1				2
80.0-81.9						1							1
82.0-83.9									1				1
Totals		3	4	12	22	44	40	25	16	15	10	9	200

the inbred zygospores produced by lateral conjugation will be more or less correlated than the cross bred zygospores produced by scalariform conjugation so far as length and diameter are concerned.

The value of perfect correlation as measured by the constant (r) is unity, while absence of correlation allows the value to become zero. Length is taken as the subject class (y) and diameter (x) as the relative class in the accompanying tables (VI and VII).

TABLE VII

CORRELATION BETWEEN LENGTH AND DIAMETER OF 200 ZYGOSPORES OF *Spirogyra inflata* (VAUCH.) PRODUCED BY SCALARIFORM CONJUGATION

	Diameter x												Totals
	23.0-23.9	24.0-24.9	25.0-25.9	26.0-26.9	27.0-27.9	28.0-28.9	29.0-29.9	30.0-30.9	31.0-31.9	32.0-32.9	33.0-33.9	34.0-34.9	
38.0-39.9													0
40.0-41.9													0
42.0-43.9													0
44.0-45.9													0
46.0-47.9					1		1				1		3
48.0-49.9						1		1	1				3
50.0-51.9			2		2	1	1						6
52.0-53.9	1		1	1	2	5	4	2					16
54.0-55.9			2	2	4	6	4	1		1			20
56.0-57.9			1		2	5	5	5	4	3			25
58.0-59.9				2		4	5	13				1	25
60.0-61.9			1			1	9	7	7	1			26
62.0-63.9			1		2	6	6	3	6		1		25
64.0-65.9				1	2	3	4	2	4	2			18
66.0-67.9					1	3	1	3	1	1	1		11
68.0-69.9				1		2	1	1	2				7
70.0-71.9					1	3	1			1			6
72.0-73.9						1	1	1	2	1			6
74.0-75.9								1		1			1
76.0-77.9						1							1
78.0-79.9					1								1
Totals	1	0	3	9	13	38	46	46	30	9	4	1	200

While one might infer that the longer a zygospore the greater the diameter, such a condition is not apparent by mere inspection of the tables in either case. Consequently on solving the equations we are prepared to find that the coefficients have an extremely low value in each group.

Lateral Conjugation	$r = .1894 \pm .0460$
Scalariform Conjugation	$r = .0934 \pm .0473$

Although in lateral conjugation the value is more than four times the probable error, one is scarcely prepared to state that there is greater correlation between characters in close breeding than in cross breeding on the basis of the data noted above. When considered with the results presented in Table XII, the conclusion seems fully established, however.

4. Comparative Size of Zygospores

The term "size" as noted in the subsequent discussion is open to various interpretations dependent as to whether length, diameter or volume is being considered, a condition which to some extent complicates the interpretation of size characters among multicellular organisms which are in general dependent on the number rather than the dimensions of the component cells.

Those zygospores produced by lateral conjugation (close bred), so far as the present material is concerned, have an average length considerably exceeding those produced by scalariform conjugation (cross bred) while the diameter is approximately the same. This is illustrated in Table VIII.

TABLE VIII

COMPARATIVE LENGTH, DIAMETER AND VOLUME OF ZYGOSPORES PRODUCED BY LATERAL (CLOSE BRED) AND BY SCALARIFORM (CROSS BRED) CONJUGATION

Method Produced	Mean Length	Mean Diameter	Mean Volume
Lateral conjugation.....	62.380 m. $\pm .178$	29.660 m. $\pm .105$	28,733 cub. m.
Scalariform conjugation.....	60.440 m. $\pm .135$	29.725 m. $\pm .080$	27,972 cub. m.
Differences favoring lateral conjugation.....	+1.940 m.	-.070 m.	+771 cub. m.

Consequently, here, the average zygospore produced by lateral conjugation has a greater volume than that produced by scalariform conjugation. Utilizing the formula

for computing the volume of a prolate spheroid ($V = 1/6\pi ld^2$) the difference is 771 cubic m. in favor of the former, although relatively this approximates only 3 per cent.

A question of some interest is at once suggested, namely, the possibilities for nourishment and development in cells of large and of small volume, inasmuch as one with a maximum volume has relatively less surface through which nourishment may be obtained. Thus growth may be retarded.

III. DISCUSSION OF RESULTS

The close bred forms on the basis of the characters studied in the given population have been found more variable as to both length and diameter, more highly correlated, and larger taking into consideration length and volume. The value of the conclusions in their application to the solution of problems of evolution is dependent on the logical application of cause and result as well as the methods of the investigation.

That the two groups of zygospores are comparatively close bred and cross bred will scarcely be denied, particularly when it is remembered that in lateral conjugation nearly all adjacent pairs of cells in a filament had united in the process, each pair producing a zygospore, all pairs having originated from the same cell. With the material taken from a part of a mass a few centimeters square, a sample of a whole population has been utilized, and from what is known of the reproduction of *Spirogyra*, it may be assumed with reasonable certainty that the entire mass had its origin from zygospores produced in a few filaments the preceding year. With practically all zygospores measured in each filament, the criticism that isolated zygospores of mixed descent were studied, and that greater variability would be expected in those produced by lateral conjugation, loses its force. Furthermore it is believed that all investigations thus far

made, upon analysis support the direct conclusions which follow.

It may be objected that cells of mature filaments originating from the zygospores should have been studied. While this would have been of interest, the zygospores themselves are individuals in the cycle of development, and the differences as represented in the groups chosen can not be said to have less value than data from another part of the life cycle.

The possibility of the results being affected by the swelling of zygospores due to the 2 per cent. formalin used in preservation, became apparent when other duties prevented measurements within the anticipated time. The first series of 358 zygospores was measured between April 2 and May 16, while the remaining 42 were measured between August 17 and 21. The question seemed an important one, and in order to test the extent of such an error if present, the average diameter of the last lot was compared with that of the first lot, the values being 29.15 m. and 29.08 m., the difference of 0.07 m. being well within the limits of the probable error. The 42 zygospores measured August 17-21 happened to consist of an equal number of lateral and scalariform individuals, which would thus tend to eliminate an error should it have occurred. Consequently the use of the formalin does not appear to have affected the results.

Some evidence has been presented that new phylogenetic characters are more variable than older characters. Thus if lateral conjugation was a recent acquisition the greater variability might have been expected. Pearl and Clawson ('07) found a higher variation in the great chela of the crayfish, *Camburus propinquus* Girard, than in the protopodites of the 2 and 3 legs, nevertheless they preferred to attribute the result to ontogenetic rather than to phylogenetic factors. MacDougall, Vail and Shull ('07) stated that

the greater variability of phylogenetically new characters as compared with older ones . . . is confirmed . . . (p. 89).

The conclusion is open to objection inasmuch as they were comparing a hybrid with a single parental type and in general the greater variability would be expected. Consequently even admitting that lateral conjugation has been a more recent development than scalariform conjugation, it would not be demonstrated that an error had thus arisen.

1. Comparative Variability

Within the limits of the characters studied so far as the present material is concerned, it is evident that the zygospores produced by close breeding are more variable than those produced by cross breeding. While it is another proposition to extend the conclusion and insist that organisms produced asexually, by pure lines, or by close breeding, are more variable than those produced sexually or by cross breeding, it would seem that the facts strongly support such a conclusion and in connection with the evidence afforded by the investigations of Warren, Casteel and Phillips, Kellogg, and Wright, Lee and Pearson, it certainly may be denied that amphimixis or cross breeding as compared with other types actually produces variations, as has long been the prevalent belief.

The question here of particular interest, however, is that of the excess type of variability represented in *Spirogyra*. Inasmuch as the material was homogeneous in every way, it may be asserted that the greater variability exhibited by the close-bred forms is not fluctuability due to environment. It is also evident that, theoretically, cross breeding produces a greater number of combinations than inbreeding, nevertheless that the variability thus resulting is overwhelmed by that of another type in nature, is clear from the results noted in the preceding pages. An excellent demonstration of such condition is obtained by recalculating constants obtained by Hayes ('12) as shown in the accompanying table (Table IX) based on data obtained in connection with the breeding of *Nicotiana tabacum*.

TABLE IX

COMPARISON OF VARIABILITY OF *Nicotiana tabacum* IN COMBINED PARENTAL TYPES (No. 3 AND 8) WITH VARIABILITY IN SEPARATE PARENTAL TYPES (No. 1, 2, 6 AND 7), IN THE FIRST HYBRID GENERATIONS (No. 4 AND 9) AND IN THE SECOND HYBRID GENERATIONS (No. 5 AND 10). No. 3 AND 8 CALCULATED FROM DATA BY HAYES IN TABLES NOTED. OTHER CONSTANTS AS GIVEN BY HAYES

No.	Table	Type	Character	S. D.	E	C. V.	E
1	XV	401	Number of leaves	0.96	±.037	5.00	±.189
2	XVI	403	" "	1.49	±.058	5.27	±.578
3	XV-XVI	401+403	" "	4.70	±.129	19.55	±.129
4	XVII	403×401	" "	1.30	±.056	5.51	±.215
5	XVIII	403×401-1-F ₂	" "	2.24	±.103	9.40	±.551
6	XV	401	Height of plant	3.85	±.150	7.00	±.150
7	XVI	403	" "	4.55	±.177	5.98	±.177
8	XV-XVI	401+403	" "	11.31	±.311	17.35	±.312
9	XVII	403×401	" "	4.54	±.177	6.41	±.249
10	XVIII	403×401-1-F ₂	" "	7.22	±.333	13.60	±.333

Here the constants of No. 3 and No. 8 have been obtained by combining the two parental types (401 and 403) both for the number of leaves and the height of the plant, and it may be noted that the coefficient of variation has dropped from 19.55 to 9.40 in the one case and from 17.35 to 13.60 in the other case. Thus variability as measured statistically has decreased. Those who have advocated an increased variability as the result of hybridization are correct when comparison is made of the F₂ generation with the F₁ generation or with a single parental generation. They are not correct, however, in making a general statement that cross breeding increases variability since the variability of the group composed of both parental types must be considered and upon so doing, it may normally be found that there has actually been a decrease in variability.

The possibility exists however that the variability will appear to have been increased when forms having the same phenotype but different genotypes are bred together. Such a condition may be illustrated by the two strains of white sweet peas crossed by Bateson which produced purple flowers in the first (F₁) generation, and purple,

pink, mixed and white flowers in the second (F_2) generation. New combinations had arisen, but only as an expression of that which already existed in the phenotypes, for there is no evidence of an increase in unit characters nor was there an actual increase in variability.

There are only three papers of a statistical nature in which it has seriously been asserted that cross-bred forms or conjugating forms produced greater variability than resulted in close-bred forms or non-conjugating forms.

The first is that of Castle, Carpenter, Clark, Mast and Barrows ('06) based on a series of observations as to the effect of cross breeding and close breeding on the variability and fertility of the small fruit fly *Drosophila ampelophila* Loew. In conclusion it was stated that "inbreeding did not affect the variability in the number of teeth of the sex comb of the male, nor the variability in size," the first opinion resulting from the value of the coefficient of variation in the number of tibial spines, the second from the standard deviation in the length of the tibia. In the former case the data certainly do not permit a clear conclusion one way or the other. In the second case, however, if the value of the coefficient of variation is computed for the length of the tibia—which, strange to say, was not done in the original investigation—and thus allowance made for the greater length of

TABLE X

ILLUSTRATING COMPARATIVE VARIABILITY OF CROSS BRED AND INBRED FORMS OF *Drosophila* AFTER COMPUTING THE VALUE OF THE COEFFICIENT OF VARIATION FOR THE LENGTH OF TIBIA FROM DATA BY CASTLE AND OTHERS

S.D. = Standard Deviation. C.V. = Coefficient of Variation.

Group		Spines of Sex Comb		Length of Tibia	
Generation	Number	S. D.	C. V.	S. D.	C. V.
Cross bred (X-1).....	100	1.749 ± .083	16.23	1.461 ± .070	3.531 ± .168
Inbred (M-31).....	100	1.568 ± .075	15.51	1.723 ± .082	4.452 ± .212
Inbred (N-30).....	100	1.684 ± .080	17.38	2.842 ± .136	8.167 ± .389
Inbred (A-61).....	100	1.857 ± .089	17.60	2.041 ± .097	5.245 ± .250

tibia in the cross-bred forms (Table X) the average variability of the three inbred groups is 68 per cent. greater than that of the cross-bred group. Consequently, the results decidedly support the facts in the present paper.

The remaining papers are those of Jennings ('11 and '13) in a study of *Paramecium*. In the first paper the breeding experiments are summarized as follows:

The progeny of conjugants are more variable, in size and in certain other respects, than the progeny of the equivalent non-conjugants. Thus conjugation increases variation.

It seems difficult to account for this conclusion if one subjects the data to a critical review. So far as a "pure race" is concerned the non-conjugants and their progeny were decidedly *more variable* than the conjugants and their progeny (Table 28, p. 94), although the small number utilized March 31 for the statistical work (42 and 34) is not sufficient to justify a conclusion in either direction. Even in a "wild culture" (Table 32, p. 99) the evidence is too conflicting to justify a definite expression of opinion. Of the seven comparisons here made among the progeny, five showed an excess variability for the conjugants, but in only one case did the difference exceed three times the probable error, while two cases showed an excess variability for the non-conjugants, the difference in one case exceeding twice the probable error. Data from numbers so small (22-95) can scarcely be considered reliable. The comparison of the variability of "all pairs" and "all unpairs" on June 22 and June 23 denotes an excess variability for those completing conjugation at the beginning of the experiment.

In the second paper Jennings concluded (p. 363) that conjugation increased the variation in the rate of reproduction. The variation was increased, but the explanation of such increase seems comparatively simple when it is noted that among the conjugants there were many with a low rate of fission with death occurring. As com-

pared with the more normal rate of fission among non-conjugants, this could result in nothing but an increased variability, having, however, no bearing on the question at issue.

At the present time, therefore, it would seem that the preponderance of evidence demonstrates that variability is decreased in cross breeding.

2. Comparative Size

The zygospores produced by close breeding have a mean length of $62.38 \mu. \pm .18 \mu.$ with a mean diameter of $29.66 \mu. \pm 10 \mu.$ and those produced by cross breeding have a mean length of $60.44 \mu. \pm .13 \mu.$ with a mean diameter of $29.725 \mu. \pm .08 \mu.$ Thus so far as length is concerned the close bred zygospores are relatively 3.2 per cent. larger and although slightly smaller in diameter, when volume is considered by utilizing the formula ($V = \frac{1}{6}\pi ld^2$) the close bred forms are also 2.8 per cent. larger. Since these results are not in accord with the general belief that cross fertilization increases size and vigor, terms which have a diverse usage, however, it will be well to consider other evidence bearing on the problem with a view of attempting an explanation which may meet the conditions imposed.

Pearl ('07) in studying the conjugation of *Paramecium* with particular reference to assortative mating, notes that "the conjugant individuals when compared with the non-conjugant, are shorter and narrower" and stated in accordance with Calkins ('02) that the reduction in size was quite probably dependent on functional changes connected with reproduction. In *Spirogyra*, however, both the close-bred and the cross-bred zygospores go through similar reproductive processes in consequence of which one may question the theory that the method of conjugation is the decisive factor in bringing about the result even in *Paramecium*.

Jennings ('11) in comparing the size of conjugant and non-conjugant *Paramecium* stated that

The progeny of conjugants . . . were a little larger than the progeny of non-conjugants and the difference appears to be significant.

This conclusion was based on measurements of length and diameter, the volume not being computed. When this is done as shown in the accompanying table (Table XI) by

TABLE XI

COMPARISON IN SIZE OF CONJUGANT AND NON-CONJUGANT FORMS OF *Paramecium aurelia* AND THEIR PROGENY BASED ON VOLUME
($V = 1/6\pi ld^2$) FROM LENGTH AND DIAMETER MEASUREMENTS BY JENNINGS, 1911

Experiment			Non-conjugants and Progeny				Conjugants and Progeny				Non-conj. Exceed the Conjugants in Volume	
Culture	Date, 1908	No	Length Microns	Diam. Mi- crons	Volume Cub. Microns	No.	Length Microns	Diam. Microns	Volume Cub. Microns			
a	N/2- Table 28	Mar. 31	34	144.59	34	87,518	48	136.95	35.52	90,471	—	2,953
		Apr. 10	65	137.97	44	139,859	61	148.20	42.30	138,844	+	1,015
		Apr. 20	103	156.48	43.82	157,327	108	160.85	42.04	148,849	+	8,478
b	C ₂ - Table 29	Sept. 16	110	132.18	?	?	138	121.91	?	?	+	?
		Sept. 18	70	116.17	31.20	59,211	15	128	31.20	65,241	—	6,030
		Sept. 26	52	122.15	34.81	77,500	11	112.36	29.50	51,198	+	26,302
c	g- Table 30	Sept. 27	118	135.35	?	?	174	118.28	?	?	+	?
		Sept. 29	10	156.40	49.60	201,465	6	135.33	36	91,833	+	109,633
	
d	k- Table 31	Sept. 12	100	140.20	?	?	336	129.58	?	?	?	?
		Oct. 28	10	136	37.60	100,673	39	131.38	35.49	86,644	+	14,029
		Oct. 30	28	123.71	34.14	75,497	25	128.16	36.32	88,520	—	13,023

utilizing the formula $V = 1/6\pi ld^2$, thus allowing for slight decreases in diameters, the facts present a different interpretation.

Three (a, b, c) of the four experiments dealing with a "pure race" of *P. aurelia* indicate that the progeny of the non-conjugants become larger, even when as a group they are smaller (a, b?) at the beginning of the experiment. While the fourth (d) indicates a reverse condition so far as the measurements of October 30 are concerned, the measurements of the sixth and seventh generations immediately preceding, demonstrate that the non-conjugants were larger. The result on October 30, where the non-conjugants became smaller, may have depended

on the elimination suggested by "all existing progeny." The extraordinary diminution in length (140 μ . to 123.71 μ .) suggests some disturbing factor of metabolism.

The results of the experiment with a "wild culture" where progeny of "unpaired" and "paired" forms of *P. caudatum* (?) were considered, again suggested to Jennings the greater size of the progeny of the paired individuals (conjugants), a condition which was particularly evident in the first generation. But it must be noted that the disturbance of the function of conjugation in "unpairing" may have produced the result. The progeny of the "unpairs" were relatively becoming larger from the first to the seventh generation. These facts taken together with the absence of measurements of mean diameters by which to calculate the mean volumes, suggest that such a conclusion based on that part of the work could not be accepted, and that the data strongly support the proposition directly contrary to Jennings that the progeny of conjugants tend to become smaller than the progeny of non-conjugants although the latter may be larger directly after conjugation as a result of slower fission. Thus the evidence from various sources, although incomplete, suggests that cross-bred unicellular organisms are smaller than close-bred forms.

Among multicellular organisms however it has long been recognized that hybrids usually grew to a larger size than either parental form, as has been observed by Kohlreuter ('63), Knight ('99), Gärtner ('49), as well as Darwin, Mendel and others, although the cause of the increased growth has been purely conjectural. It is quite evident that the result is due to either the increased number of cells, a suggestion made by East, to the increased size of the cells, or to the combination of both conditions.

The question immediately arises as to the cause of the increased size and vigor among cross-bred multicellular organisms when the evidence indicates that cross-bred unicellular organisms are smaller instead of larger.

Some investigations in progress³ suggest an answer meeting the conditions, although more than a provisional opinion may as yet not be ventured. This is to the effect that the cells of cross-bred multicellular organisms are actually smaller than the cells of pure line or inbred organisms, and that the more rapid division is a function of the greater ratio surface has to volume in a small cell with the better opportunity this afforded for an increased metabolism.

The increase of size in plant and animal forms to the physiological limit has great importance for the future of agriculture and stock breeding, but many subsidiary problems must be solved before practical results are attained in this direction. The relative rate of growth, number and size of the constituent cells of pure line and of hybrid individuals is one of the problems.

3. *Comparative Correlation Resulting from Close Breeding and Cross Breeding*

The close-bred zygospores are more correlated as to length and diameter than the cross-bred zygospores, but since the difference only slightly exceeds twice the probable error, the value of the result here is questionable. Considering other investigations (Table XII), it may be noted that the group containing close-bred, asexual or non-conjugating organisms, is more highly correlated in respect to characters than the group consisting of cross-bred, sexual, or conjugating organisms, although two exceptions, No. 12 and No. 14, are presented. An interesting fact, although possibly only a coincidence, is that cross bred zygospores of *Spirogyra* and of conjugating *Paramecium* have approximately only one half the correlation exhibited by close bred zygospores of *Spirogyra* and by non-conjugating *Paramecium*.

The explanation of the conclusion here reached, that the value of a character "*x*" in cross-bred forms does not have the same tendency to change that the value of a

³ Walton ('14).

TABLE XII

COMPARATIVE CORRELATION OF CHARACTERS IN CROSS-BRED AND CLOSE-BRED ORGANISMS INCLUDING CONJUGANT AND NON-CONJUGANT *Paramecium*, AND PARTHENOGENETIC AND SEXUALLY PRODUCED WASPS

	Organism	Authority	Characters Compared	Type of Development	Value of "y"
1	<i>Paramecium</i>	Pearl, '07	Length and diameter.	Non-conjugants, Ser. A	.589 ± .03
2	"	" "	"	Conjugants.	.278 ± .04
3	<i>Drosophila</i>	Barrows, '06	Number of spines and length of tibia.	Close bred, Ser. A-61.	.469 ± .05
4	"	" "	"	" " Ser. M-31.	.448 ± .05
5	"	" "	"	" " Ser. N-30.	.708 ± .03
6	"	" "	"	Cross bred, Ser. X-1.	.141 ± .07
7	<i>Nicotiana</i>	Hayes, '12	Number of leaves and height.	Close bred, No. 401.	.368 ± .05
8	"	" "	"	" " No. 403.	.631 ± .03
9	"	" "	"	Cross bred, 403 × 401.	.406 ± .05
10	"	" "	Length and breadth of leaf.	Close bred, No. 401.	.684 ± .03
11	"	" "	"	" " No. 403.	.497 ± .04
12	"	" "	"	Cross bred, 403 × 401.	.818 ± .02
13	<i>Vespa vulgaris</i>	Wright, Lee,	Length and breadth of r. wing.	Drones.	.772 ± .02
14	"	and	"	Workers.	.912 ± .01
15	"	Pearson, '07	"	Queens.	.558 ± .04
16	<i>Spirogyra</i>	Walton, '15	Length and diameter.	Close bred (Lat. C.)	.189 ± .05
17	"	" "	"	Cross bred (Seal. C.)	.093 ± .05

related character "y" has in close-bred forms, apparently rests on a Mendelian basis. Its importance in evolution, beyond the idea that more pronounced temporary combinations are thus allowed in the trial and error plan of nature, is conjectural.

4. *Amphimixis and Death*

With the assumption that the results obtained in the preceding investigation, together with the data presented by other writers, when correctly analyzed, strongly supports the view that asexually produced organisms tend to be more variable than those produced by the union of two gametes, there is furnished evidence for the interpretation of the origin of sex—amphimixis and also for the origin of death that would seem to rest upon a much more secure basis than the purely speculative theories of Weismann, Nägeli, Hatchescheck, Metschnikoff, Minot, etc., which have previously been advanced.

The chief advantage gained in the reduction of variability, while somewhat conjectural, would appear to be

that of holding organisms within limited bounds, or in other words, *asexually produced organisms in general tend by their variability to exceed the limits of their environment and thus perish, while organisms produced by the mingling of two diverse lines of germ plasm with their lessened variability meet the conditions of the comparatively slowly changing environment and their race persists*. This idea was proposed entirely upon speculative grounds by Hatscheck ('87) who suggested that variation would run riot if not controlled by the union of germ cells, and it would now appear that the facts support such a proposition. While it has been suggested that the chief function of amphimixis was that of rejuvenation, a consideration of the discussion on "Comparative Size" as well as the recent experimental results obtained in the production of *Paramecium* do not support such an opinion to the exclusion of the hypothesis here put forward. East and Hayes ('12) have advanced the theory that recombinations in accordance with Mendelian principles were the chief purpose of amphimixis. While new combinations are thus brought about, apparently there exists a real difficulty in understanding how transitory heterozygotic forms could become of selective value in originating and maintaining such a process.

The acceptance of the conclusion that asexually produced organisms are more variable than those produced by amphimixis, and that thus some of the units are more readily subject to the eliminating influences of the environment, affords a comparatively simple explanation of the origin of death in multicellular forms which are built up of such units—the cell. Consequently the inference is that⁴ *death occurs as the result of the continually forming body cells becoming so variable through the absence of control by amphimixis, that eventually some one group fails to meet the limits imposed by the environment, and these together with the remainder of the colony*

⁴ Walton, *Science*, p. 216, 1909.

—*the individual—perish*. The experiments of Woodruff ('11, etc.) who in extending the work of Maupas and of Calkins was able to rear several thousand generations of *Paramecium* without conjugation, as well as the investigations of Harrison subsequently elaborated by Carrel, where human and other animal tissues maintained cell division for a prolonged time in an artificial medium, are here of much interest. In each case the result is brought about by the favorable artificial environment, and it is made more clear that death itself is wholly or in part due to the unfavorable conditions surrounding an organism.

IV. A WORKING HYPOTHESIS OF EVOLUTION

Investigations during the last fifteen years, instead of establishing evolution as the simple process of natural selection conjectured by Darwin and others, have made it evident that the results are due to many factors of much complexity. While the diversity of organisms depends on variation—their inheritance and non-inheritance—it is becoming more and more apparent that the term is too comprehensive and covers variations arising in organisms from causes quite different from one another.

The results reached in the preceding pages indicate the need of extending the older terminology as used by Plate, '13, and others where variations are separated into "somations" or fluctuations induced by the environment and not inherited, and "mutations" or blastovariations arising in the germ plasm and inherited, if a clearer understanding is to be obtained of evolution and its application. Therefore the following scheme is proposed.⁵

⁵ Several interesting groupings of variations have been suggested by Spillman, Baur and others, none of which, however, appear to meet present conditions.

VARIATIONS

- 4¹. Apparently originating in accordance with definite laws *A. Normations.*
- B*¹. Induced by general environmental stimuli, (food supply, use and disuse, etc.), but not inherited so far as evidence shows..... 1. *Fluctuations.*
- B*². Not induced by environmental stimuli; inherited.
- C*¹. Arising through the transference of factors by the combination of two ancestral lines in accordance with Mendelian principles, but exhibiting "per se" no definite progress..... 2. *Amphimutations* ("mutations" in part).
- C*². Arising through causes at present unknown, but which, from the progressive results obtained, may be assumed to originate in accordance with definite laws 3. *Cumulations.*⁶
- 4². Apparently not originating in accordance with definite laws *B. Abnormations.*
- B*¹. Induced during early developmental stages of the embryo from intracellular (?) stimuli, and inherited.
- C*¹. Arising through the abnormal segregation of the hereditary material (chromosomes?) .. 4. *Malsegregations* ("mutations" in part).
- C*². Arising by the loss of hereditary qualities.
- D*¹. Resulting from the functional loss of a factor controlling a character 5. *Defactorations* ("mutations" in part).
- D*². Resulting from the partial functional loss of a factor controlling a character.... 6. *Fractionations* ("mutations" in part).
- B*². Induced during the early developmental stages of the embryo from extracellular (?) stimuli and not inherited 7. *Malformations.*⁷

While any scheme presented must change as new facts are obtained, a terminology is of value in proportion as it gives a basis for future progress. The objection that it is not possible to point out a specific cumulation by no means indicates the absence of such progressive variations taking long intervals of time, by the haphazard

⁶ Cumulations—from cumulo, to increase—including the names of the following groups, with the exception of fractionations proposed by Bateson, are based on the apparent origin of the variations.

⁷ Many so-called malformations originate as defactorations, etc.

method of nature, in which to bring about a change evident to mankind. That the weight of evidence, so far as investigations have gone, is against evolution by means of the other variations noted, makes the explanation the more plausible. While it is true that Bateson ('14) has urged the consideration of the proposition that organic changes occur through the loss of inhibiting factors—defactorations—such a double negative theory assumes a decreasing complexity instead of an increasing complexity of protoplasm, as already pointed out by Castle, ('15) and seems impossible to maintain.

On the interpretation here presented, the diversity of organic forms is more complex than earlier imagined, and the problem of positive racial improvement is still far from solution. Loss as well as segregation factors may add new forms which really contain nothing new. To build up and not to break down is the desideratum, and the data obtained would seem to suggest that pure line breeding with the employment of statistical methods to show any progress would be the path leading most directly to the goal.

VI. CONCLUSIONS

1. *Direct Conclusions*

The following conclusions drawn from the investigation are primarily statements of fact.

1. Zygospores of *Spirogyra inflata* (Vauch.) produced by lateral conjugation or close breeding (quasi-parthenogenesis) are relatively 26 per cent. more variable in length and 31 per cent. more variable in diameter as measured by the coefficient of variation, than those produced by scalariform conjugation or cross breeding (sexual reproduction).

2. The size (volume) is greater in the average (mean) zygospore close bred by lateral conjugation, where the mean length is $62.38 \mu. \pm .178$, than in the average zygo-

spore cross bred by scalariform conjugation, where the mean length is $60.44 \mu. \pm .135$. The diameter is approximately the same in both types.

3. In zygospores produced by lateral conjugation there exists a positive correlation between length and diameter of $.1894 = .0460$, while in scalariform conjugation the value is $.0934 = .0473$. This is in general agreement with results obtained by others although here the difference is not significant when the probable error is considered.

4. In the material studied approximately 45 per cent. of the zygospores were formed by lateral conjugation, the remaining 55 per cent. by scalariform conjugation.

5. The material studied was strictly homogeneous, and evidently arose from the same parental stock, both types of filaments being intermingled with no structural differences except those of conjugation. Consequently the differences in variability are not the result of fluctuability.

2. Indirect Conclusions

The conclusions here presented are generalizations based on the present investigation as well as the work of others, and represent propositions concerning which differences of opinion may exist.

1. Amphimixis, cross-breeding, etc., decreases and does not augment variability (cumulability) although amphimutability may temporarily be increased.

2. Close bred forms are more highly correlated in respect to related characters than cross-bred forms.

3. Variations, so far as their origin is concerned, may be separated into (A) Normations consisting of (1) fluctuations, (2) amphimutations, and (3) cumulations, and into (B) Abnormations consisting of (1) malsegregations, (2) defactorations, (3) fractionations, and (4) malformations.

4. Cumulations may best be investigated among organisms produced asexually, by pure lines, or by close breeding than by cross breeding, etc.

5. Sexual reproduction and cross fertilization have been advantageous in the evolution of organisms by limiting cumulability and thus confining the progress of the group to a path bounded by the more permanent environment.

6. Death occurs as a result of the continually forming body cells becoming so variable through the absence of control by amphimixis, that eventually some one group fails to meet the limits imposed by the environment, and these together with the remainder of the colony—the individual—perish.

3. Hypotheses

The following opinions in the nature of hypotheses based to a large extent on the preceding work may be confirmed or invalidated by future investigations.

1. Variability (cumulability) will be greater in a small and isolated population than in a large and less isolated population.

2. Progressive evolution has resulted from factors arising through cumulations without reference to amphimutations (Mendelian combinations).

3. Characters once established by cumulations produce by fluctuations, amphimutations, etc., the diversity of organic life. Such secondary variations are only indirectly the products of evolution.

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GENETIC STUDIES OF SEVERAL GEOGRAPHIC RACES OF CALIFORNIA DEER-MICE¹

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SOME of those present may recall a resolution which was adopted at a meeting of the Biological Society of the Pacific, held in Berkeley, in April, 1913, endorsing a project for the study of certain problems, related both to genetics and to geographical distribution. During the same year, the Scripps Institution for Biological Research found it possible to undertake the execution of this project, and the author of the present paper was chosen to carry it out. It is my object to-day to offer a preliminary report upon the results of these studies.

To those who have been so fortunate as to work in fields which yield quicker returns than does that of experimental breeding, it may seem that something more than a "preliminary report" might reasonably be expected after the lapse of a year and a half. If any justification is needed for such seeming slothfulness, I need only remark that my studies have already necessitated the trapping of about 600 living mice, of my chosen species, in four widely distant parts of the state, together with the rearing of several hundred others which were born in captivity; and that I have made measurements of some 500 of these animals, including skeletal measurements of over 400. Care of this rather large family of pets, statistical treatment of the measurements, continuous meteorological observations at several points, and the preparation of a certain number of skins and color photographs, are also to be included in the technique of this

¹ Read before a joint meeting of the American Association for the Advancement of Science (Section F), the American Society of Naturalists, the American Society of Zoologists, the American Genetic Association, and the Eugenic Research Association, at Stanford University, August 4, 1915.

project. Without the generous opportunities afforded me by the Scripps Institution, the work could never have been undertaken. And of an importance only second in order I must mention the assistance rendered me throughout these studies by the Museum of Vertebrate Zoology at Berkeley.

The resolution to which I have referred above formulated four questions which were regarded as especially worthy of consideration in the investigations contemplated. These were:

1. To what extent do influences such as external conditions, the exercise of organs or faculties, etc., which produce modifications of structure or function in the parent, result in bringing about parallel changes in the offspring?

2. If such changes are, in reality, found to reappear in the offspring, do they constitute true examples of heredity?

3. Are the subspecies or geographical races of the systematic zoologists fixed, in the sense of being hereditary, or do the differences by which they are distinguished depend upon conditions which must act anew during the lifetime of each individual?

4. If these subspecific characteristics are actually found to "breed true," do they owe their existence at the outset to "mutations" or to the cumulative effect of environmental influences, or to the mere fact of isolation, acting in some way independently of those influences?

To a large section of experimental breeders in this country, to whom "genetics" is synonymous with Mendelism, such a formulation of problems as this doubtless seems hopelessly archaic. "What is the use of raising all these dead issues," they will ask, "as if Weismann and De Vries and Johannsen had never lived?" And as for the question of subspecies, I suspect that some of our critics would grant them no existence whatever, outside the overwrought imagination of certain taxonomists.

Those, however, who have read dispassionately such able compilations of evidence as are offered us, for

example, by Plate² and Semon³ are not likely to fall into the shallow dogmatism which dismisses the whole "acquired characters" question as once for all settled. And those who have taken the trouble to carefully examine a few trays of specimens, representing the subspecies of some widely ranging bird or mammal, will not so readily resort to a subjective interpretation of the phenomenon of geographic variation.

I shall give chief attention to-day to the case of a single species of white-footed mouse, or deer-mouse of the genus *Peromyscus*. According to Osgood,⁴ the chief monographer of this genus, the species *maniculatus* comprises about 40 distinguishable geographic races, many of which are so unlike that they would be given full specific rank but for the fact that they intergrade insensibly with one another.

My own special studies have had to do chiefly with those subspecies of *Peromyscus maniculatus* which fall within the limits of the state of California. The first investigations have naturally been directed toward a careful examination of mice representing each of these local races, together with a determination, so far as possible, of the meteorological conditions to which they are subjected in nature. A search for correlations of any sort between structural and environmental differences was, of course, early undertaken.

Mice were collected at four points within the state: Eureka, Berkeley, La Jolla, and in the Mojave Desert near Victorville. At Eureka, Berkeley and Victorville, self-recording instruments (thermographs and hygrographs) have been left in charge of assistants for nine to fifteen months, and recording instruments will be installed at La Jolla this summer. It is planned to continue these records for at least two years. The instru-

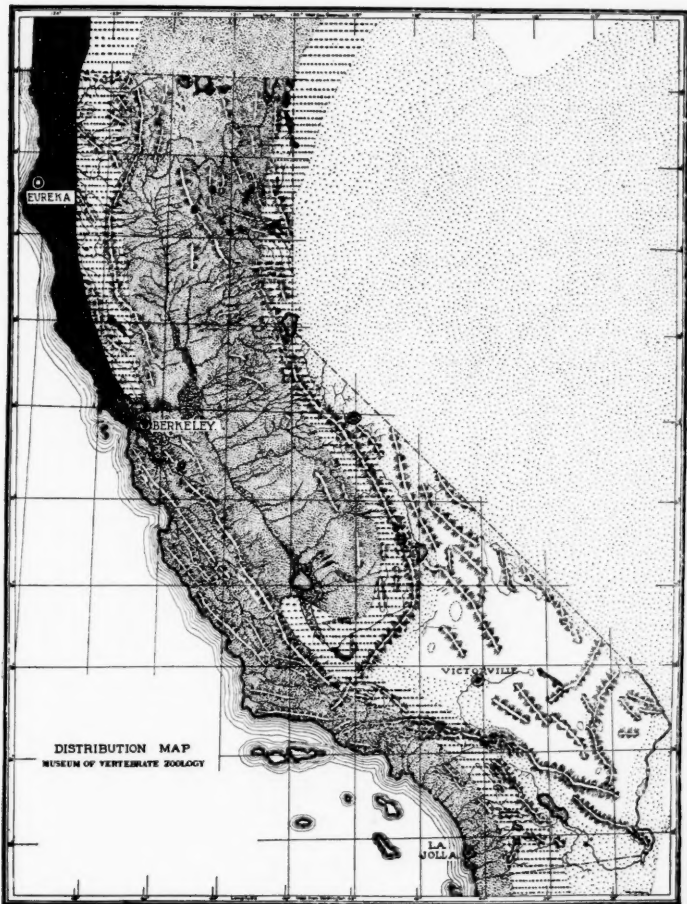
² "Selektionsprinzip," vierte Auflage, Engelmann, 1913.

³ "Das Problem der Vererbung 'erworbener Eigenschaften,'" Engelmann, 1912.

⁴ "Revision of the Mice of the American Genus *Peromyscus*," U. S. Department of Agriculture, Biological Survey, 1909.

ments are placed in positions more nearly representing the natural environmental conditions of the animals than is customary for regular Weather Bureau stations (*e. g.*, in a redwood forest on the outskirts of Eureka).

The Eureka mice are assigned to the subspecies "*rubidus*," those from the desert to "*sonoriensis*," while those



DISTRIBUTION OF THE SUBSPECIES OF *Peromyscus maniculatus* IN CALIFORNIA AND NEVADA, BASED UPON THE DISTRIBUTION MAP OF OSGOOD (1909). The heaviest shading denotes the range of *P. m. rubidus*, the intermediate shading that of *gambeli*, the lightest that of *sonoriensis*. Areas of intergradation between two races are indicated by dotted lines.

from Berkeley and La Jolla are assigned by Osgood to the same subspecies "*gambeli*," although, as I shall point out, there are certain slight differences of type between the two.

Now, as to characters, I have made 14 measurements of each completely measured mouse. Certain color characters, not capable of quantitative expression, have also been taken into consideration. I shall first consider the measurable parts. I must introduce this discussion by stating that my comparisons are entirely between animals of the same body length. When I say that *P. m. rubidus* has a longer tail than *sonoriensis*, I mean that this is true for mice of equal size. Owing to the impracticability of giving you a mathematical justification of all the steps which I have taken, I will ask you to credit me with a knowledge of the more elementary statistical methods.⁵ I must also explain that I have thus far failed to kill and measure many animals from which I shall before long have full data. At present these are being retained for breeding purposes. Hence my series of measurements, in certain cases, is very small.

To present these subspecific characters briefly, I may say that, in respect to tail and foot length, *rubidus* stands in a class by itself. It does not require the trained eye of a systematist to detect the fact that this northern race has conspicuously longer tail and feet. In the case of the tail, this difference is due almost wholly to a difference in the length of the individual vertebræ, not to an increase in the number of these.⁶ The other three races (*sonoriensis* and the two lots of *gambeli*) show no statistically certain differences in either of these characters. *P. m. rubidus* likewise has a significantly greater skull length and probably also a greater cranial capacity.⁷

⁵ The detailed data must be deferred until a somewhat later stage of the work. Some of these were presented to the meeting in the form of graphs.

⁶ The same was found to be true of the artificially induced modifications in tail length, described by me elsewhere for white mice.

⁷ Determined by suitably cleaning and then desiccating the skulls, and weighing the volume of mercury which just filled the cranial cavity.

The La Jolla race of *gambeli* seems to fall second in the list in this regard.

The only significant difference in ear length is that between the two races of *gambeli*, the La Jolla stock having noticeably longer ears than the Berkeley stock, while *rubidus* and *sonoriensis* appear to be intermediate in this respect.

As regards color differences, these relate chiefly (1) to the depth of shade, and (2) to the extensivity of the pigmented areas. A careful comparison of large numbers of the Berkeley race (*gambeli*) and the desert race (*sonoriensis*) revealed at least ten recognizable differences of this class, though in many cases these were merely different expressions of the same fundamental difference. None of the distinctions between these two races are absolute ones, holding between any two individuals of the contrasted races. Rather they are distinctions "on the whole," expressed by differences of mode or mean. Taken collectively, however, it is likely that these characters form an ensemble sufficiently distinct to reveal the identity of practically every specimen.

The most widely separate of the races, in respect to color, are *rubidus* and *sonoriensis*, the former race being very much darker than the latter. The two lots of *gambeli* occupy intermediate positions between the others.

METEOROLOGICAL DATA AT FOUR CALIFORNIA STATIONS.

	Temperature (°F.)		Humidity		Rainfall
	Annual Mean	Annual Range	Annual Mean	Daily range	Annual total
Eureka ⁸	51.6	8.9	86	Very small	45
Berkeley.....	56.1 ⁹	14.4 ⁹	83 ⁸	Small	26 ⁹
La Jolla (San Diego) ⁸	60.6	14.7	75	Small	10(-)
Mojave Desert.....	(Mojave) ⁸ 63.6	(Mojave) ⁸ 40.4	(Victorville) ¹⁰ 45±	High	(Victorville) ¹¹ 6

⁸ U. S. Weather Bureau.

⁹ University Observatory.

¹⁰ Computed from observations during present experiments (only one half year at this station).

¹¹ Rain-gauge records of Mr. Reginald Frost.

Now do we find any instances of correlation between these differences of structure or color and differences in environmental conditions? The most conspicuous of the structural differences relate to the greater length of the tail and foot of the Eureka race (*rubidus*) as compared with any of the other three races here considered. It is of considerable interest to note that there is here an increase in the length of these appendages as we pass to the northward, a circumstance which is still further emphasized by the condition of certain Alaskan subspecies. In fact, so far as these coastal subspecies of *Peromyscus* are concerned, there seems to be, within certain limits, a reversal of Allen's principle of the shortening of "peripheral parts" as we pass from south to north. The facts here revealed are likewise out of harmony with my own experimental results from white mice, which showed conclusively that low temperature and high humidity led to a decrease, rather than an increase in the length of the tail and foot.¹² A little later I shall point out the pronounced effect of certain other artificial influences upon the length of these appendages in *Peromyscus*, though I must admit that these later experimental results furnish no more satisfactory clue to the origin of these differences in nature.

On the whole, then, these preliminary researches do not offer much ground for believing that the differences found in the tails and feet of these wild races of *Peromyscus* result directly from any differences in environmental stimuli, or for expecting that they will respond appreciably to artificial climatic changes.

Passing to color differences, we seem to have here a good illustration of that correlation between atmospheric humidity and depth of pigmentation which has long been recognized to hold for mammals, birds and some other animals. If we arrange our four environments in ascending order with respect to their atmospheric humidity

¹² Cf. *Journal of Experimental Zoology*, April, 1915, and earlier papers therein cited.

(the same order holds with respect to their rainfall), we have the series: (1) Victorville, (2) La Jolla, (3) Berkeley and (4) Eureka. Correspondingly, the desert mouse (*sonoriensis*) is the palest of the lot, while the La Jolla mouse, the Berkeley mouse and the Eureka mouse follow in the order of increasing pigmentation. This relation, when viewed in connection with a wide range of known facts, and with certain experimental data to be noted later, can hardly be regarded as accidental. Any exact quantitative determination of the density of pigmentation would of course be difficult, and it has not yet been attempted. But the width of the dorsal median stripe of the tail is found to serve in some measure as an index of the extension of the darkly pigmented areas. It is interesting to note, in order of increasing width: *sonoriensis* (28 per cent.), *gambeli* (32 per cent.) and *rubidus* (43 per cent.). (The *gambeli* considered are from La Jolla.)

Let us grant then, provisionally, some sort of causal relationship between atmospheric humidity and the quantity of pigment in the hair or feathers. Now, aside from our ignorance of the physics and chemistry of the processes here involved, there is still a most important biological question left unsolved: Are these differences in pigmentation between the various geographical races germinal in their origin or are they purely somatic and individually acquired?

It was but a few years ago that Mr. J. A. Allen¹³ was shocked by the very moderate suggestion of President Jordan's¹⁴ that perhaps some of our subspecific differences were "ontogenetic," and not racially fixed. Mr. Allen was shocked, though unable to offer any really substantial evidence in reply. In this uncertainty over so elementary a matter of fact, everybody suggested decisive experiments for some one else to perform, but somehow no one seemed disposed to perform them. At least the

¹³ *Science*, January 26, 1906.

¹⁴ *Science*, December 29, 1905.

question of the fixity of the subspecies of mammals and birds has, to my knowledge, never before been put to experimental test.¹⁵

Now, in view of the subject matter of the present paper, it would ill become me to underrate the value of such tests. But I think that I have had enough to do with the experimental method in zoology to make me realize its rigid limitations. It is seldom indeed that we are able to perform a really crucial experiment and to obtain unequivocal results. Moreover, the mills of the gods grind slowly, while the single human life is short.

I am therefore disposed to attach considerable importance to what have been called "Nature's experiments." Certain of these have been cited by Grinnell and Swarth¹⁶ in their bearing on the subspecies question. For example, two well-marked local races or subspecies of song-sparrow occur in southern California, on opposite sides of the high mountain range which divides the coastal plain from the desert interior. These races are separated nearly everywhere by the mountain barrier, but at certain points passes through the latter occur, permitting of migration from one side to the other.

Now these authors find localities in which the coast form of song-sparrow has penetrated, for considerable distances, into the desert and has become established there. Nevertheless, these invaders, which have perhaps been exposed for many generations to the desert atmosphere, have retained the darker pigmentation and other characteristics proper to the coastal plain.

Again, both Grinnell and Taylor¹⁷ have taken specimens of *Peromyscus maniculatus*, which they believe to be typical representatives of the desert race *sonoriensis*,

¹⁵ It is interesting to note that the often cited case of the Porto Santo rabbit has been recently put in quite a new light by the investigations of G. S. Miller, who finds that this transplanted race is merely the *unmodified* rabbit of southern Europe. (Catalogue of the mammals of western Europe, in the British Museum, London, 1912.)

¹⁶ University of California Publications in Zoology, Vol. 10, No. 9, 1913.

¹⁷ University of California Publications in Zoology, Vol. 7, No. 7, 1911.

high up in the mountains of California and Nevada. These points are continuous with the main habitat of the subspecies in the desert lowlands and plateaus, in the sense that no abrupt barriers intervene, but they present very great differences in climate and vegetation.

Facts of this sort—"natural experiments," as we may call them—seem to show that these subspecific differences manifest themselves in a large degree independently of climatic conditions, in other words, that they are of germinal rather than of somatic origin.

But these "natural experiments" are not entirely conclusive, for we can never be quite certain what the actual condition are which Nature has imposed in a given case. Granting that these darker song-sparrows in the desert are actually invaders from the coastal plain, we have no means of knowing how long they have been exposed to the desert conditions. Also is it definitely known that their restricted habitat in certain portions of the desert does not agree with their original habitat in respect to those factors which are really essential in determining their characteristic coloration?

My own first attempt at transplantation consisted in bringing a considerable number of specimens of *P. m. sonoriensis* from the vicinity of Victorville to Berkeley.¹⁸ At the latter point, the mice were kept in cages, freely exposed to the air, and under atmospheric conditions as nearly natural as possible. For control, numbers of the Berkeley race were reared in neighboring cages. The result of this experiment I can state briefly: *Neither the originally introduced animals nor their offspring, nor their grandchildren, have thus far shown any perceptible approach to the local type. They are still obviously of the sonoriensis race.* If there is any tend-

¹⁸ The contrast between the climatic conditions at the two points is indicated, to a certain extent, in the foregoing table. But this does not show one of the most characteristic differences, namely, the relatively enormous diurnal fluctuations of temperature and humidity in the desert, as compared with those in the coastal region.

ency for these mice to be modified in the direction of *gambeli*, this tendency is not sufficiently great to be detected through any mere gross comparison, based upon qualitative characters. Unfortunately, these two races differ significantly in color characters only, so that an exact quantitative test of this question is not here possible. But I am now rearing *P. m. rubidus* at La Jolla, an experiment which would seem to be much more promising of decisive results than the one just described.

Enough has been done, therefore, to prove that the color differences between *sonoriensis* and *gambeli* are at least in a large degree germinal and independent of environmental influences acting during a single lifetime. Whether or not they are *wholly* germinal, and if so, whether they can resist change for an indefinite number of generations, remains to be learned. Suppose that we had a shifting of the mean to the extent of one per cent., or even ten per cent., in the direction of *gambeli*. Amid all the natural variability, we certainly should not be able to detect such a change with the unaided eye.

In interpreting the facts that I have offered as to the relative fixity of these color differences of *Peromyscus*, we should have due regard for various other experiments which show that environmental influences may produce notable changes of coloration in the lifetime of an individual. As especially comparable with these tests of my own, though differing completely in the outcome, may be cited the experiments of Beebe.¹⁹ This author produced a marked increase of pigmentation in the feathers of three species of birds by rearing them in an atmosphere of abnormally high humidity.

Beebe's experiments seem to show that pigmentation may in some cases be altered during a single lifetime by changes of humidity. The generally known facts of geographic distribution show that there is in nature a distinct correlation between pigmentation and humidity. My own experiments show that these geographic differ-

¹⁹ *Zoologica*, N. Y. Zool. Soc., Sept. 25, 1907.

ences of pigmentation are capable of being relatively fixed germinally. Bringing these three sets of facts together, they are, in my opinion, most readily harmonized on the assumption that the direct effects of humidity upon the organism may finally become fixed through heredity.²⁰ But I grant that this assumption is as yet far from proved.

A study of the effects of captivity forms a new line of inquiry which was hardly considered when the present researches were undertaken. For some reasons, however, this line of investigation now seems quite as promising as the search for climatic effects. In the first place, my comparatively meager results in this field already reveal striking differences between the wild stock and the individuals of the first generation reared in captivity. These differences relate to absolute size (the domesticated generation being smaller), to length of tail and foot (both shorter in the domesticated) and to the length of the femur and the pelvis, which differ in the same direction. In the case of the femur, in particular, the differences are striking, even upon the most casual inspection, and they are absolutely certain statistically. They hold for both sexes and for both of the subspecies which have thus far been tested in this regard. Thus far, no certain effects upon the cranial capacity of the captive lots have been detected.

Such differences as those found probably have nothing to do with the direct effects of external conditions, but are due to the *activities* of the animal. For this reason, they are of much higher value as a test of the Lamarckian principle, which could help us most in explaining the perfecting of active parts through use or of their degeneration through disuse.

The experimental results just referred to suggested the possibility that some of the differences found to occur in nature might have had a functional basis. The larger

²⁰ Whether this results from "somatic" or "parallel induction" need not concern us here.

feet and tail of *P. m. rubidus*, for example, might be attributed to its (assumed) greater activity. Unfortunately for this theory, the pelvis and femur of *rubidus* is no greater (slightly smaller, it would seem) than in the other races. Had the enlargement of the foot been due to greater functional activity the skeletal parts named would probably have also undergone an increase in size.

From a series of measurements upon the skeletons of wild and domestic fowls and rabbits, Darwin inferred a relative decrease in the size of the wings of the former and of the cranial capacity of the latter, under the influence of domestication. These conclusions may well be true, but the evidence offered seems to be open to several objections. (1) They are based on too small numbers and lack the precision demanded by modern statistical work. (2) We can only infer the exact nature of the wild stock from which the domesticated races are descended. (3) We can not judge of the extent to which artificial selection may have played a part in bringing about these differences. It was perhaps considerable. (4) We do not know how much of this modification results from use or disuse during the animal's own lifetime. It might be contended that the change was not congenital at all.

Lapicque and Girard have, in the main, demonstrated a smaller cranial capacity in domesticated animals as compared with wild ones, but aside from the more rigorous statistical methods employed by these authors, the biological significance of their results is open to the same objections as I have stated in the case of Darwin's.

Hatai²¹ has shown that the albino rat has a smaller brain than the wild Norway rat, when individuals of the same size are compared. This proves nothing definite, however, as to the effects of domestication, a fact which the author recognizes. The deficient brain of the albino may have been part of the same mutation which brought about the albinic condition. Or it may have resulted in part from the selection of tamer individuals. Or the

²¹ *Anatomical Record*, Vol. 3, 1909, p. 245.

difference between the two races may be purely ontogenetic. Indeed, Donaldson²² seems to have demonstrated the effects of exercise upon the weight of the nervous system of the rat.

By taking a known stock of wild mice and measuring each successive generation reared in captivity, and by being careful to avoid selection, it would seem that the foregoing ambiguities of interpretation might in a large degree be obviated. It is possible, therefore, that this phase of the subject deserves quite as much attention as the problems relating to the physical environment and the distribution of subspecies.

Hybridization has thus far failed completely between *rubidus* and *gambeli* (48 matings). The Berkeley *gambeli* has, however, been successfully crossed with *sonoriensis* and some young of an F₂ generation have already been obtained. Owing to the intergrading and widely overlapping character of the differences between these two races, it does not seem likely that they will lend themselves well to Mendelian analysis. But it would be idle for me to discuss the results of these crosses at the present stage of the experiments. Further attempts will, of course, be made to obtain hybrids between the more widely separated races.

(Since writing the foregoing, several successful matings between *rubidus* and *sonoriensis* have been effected.)

²² *Journal of Comparative Neurology*, Vol. 21, No. 2, 1911.

SHORTER ARTICLES AND DISCUSSION

ADDITIONAL EVIDENCE OF MUTATION IN *ÆNOTHERA*

IN a group of recent papers Bartlett reports on the remarkable behavior of certain wild species of *Ænothera* grown in large cultures, which behavior he regards as strong evidence for the mutation theory of De Vries. The facts are presented very clearly, but there is, however, a point of view which has not been considered in the interpretation of the conditions in his material, certain possibilities that must be reckoned with in the critical examination of such evidence. The suggestions that I shall offer will concern chiefly the genetic purity of the forms studied, a condition which is of course basic to studies on mutation as well as to Mendelian experimentation.

Ænothera pratincola Bartlett¹ is a small-flowered, close-pollinated species apparently common in the North Central States. Seven strains derived from wild mother plants at Lexington, Kentucky, gave rise to a variant, *nummularia*, which differs from the parent type in the form of the seedling leaves, foliage, pubescence of the ovary and calyx, and in the manner in which the calyx is ruptured in the opening of the flower. *Nummularia* appeared with a frequency of about 1 plant to every 400 seeds sown and 1 plant to every 250 seedlings since the germination of the seeds in the earth was 66 per cent. Two of the strains produced *nummularia* in both the F_1 and F_2 generations. Further studies will be undertaken to determine whether *pratincola* will continue to give *nummularia* or whether it may perhaps in later generations produce stable individuals. *Nummularia* develops a low percentage of good pollen (less than 50 per cent.) while *pratincola* has a high proportion (90 per cent.); *nummularia* also forms very few good seeds to a capsule, and of these only 34 per cent. are viable. Small cultures grown from *nummularia* seeds gave no plants of *pratincola*, but certain new forms appeared. The high degree of sterility both gametic and zygotic shown by *nummularia* is striking and demands study, for it will make a difference in the interpretation of the behavior of this plant whether the sterility is physiological or genetic in character.

¹ Bartlett, H. H., "Additional Evidence of Mutation in *Ænothera*," *Bot. Gaz.*, Vol. LIX, p. 81, 1915.

Cultures of *pratincta* and *nummularia* should be grown from germinations established experimentally to be complete, with records of the residue of sterile seed-like structures, and the two forms should be crossed with the purest *Oenothera* known to determine whether or not the F_1 hybrid generations are uniform. Should F_1 hybrid generations consist of distinct classes we would be justly suspicious of the purity of the stock.

A second paper of Bartlett² describes a series of cultures of *Oenothera stenomeris*, a cruciate-flowered species from Montgomery County, Maryland. Two sharply marked new types were produced by the typical form of the species, *gigas* represented by one specimen with 28 chromosomes, and *lasiopetala* with hairy petals. The *gigas* plant appeared in the F_4 generation of a line of *stenomeris*. The peculiarities separating it from *stenomeris* are similar to the distinctions between *Lamarckiana* and its derivative *gigas*. Thus both *gigas* forms are more persistently biennial in habit than their parents, both have thicker, broader leaves, stouter stems, larger buds, thicker fruits, 4-lobed pollen grains, and twice as many chromosomes. A progeny of 63 individuals from the original *gigas* plant consisted of 54 typical *gigas*, 6 narrow-leaved variants, and 3 "secondary mutations"; the form thus, as with the *gigas* from *Lamarckiana*, produces a varied offspring. Two of the "secondary mutations" were dwarfs and one had the characters of *lasiopetala*, hairy petals, and in addition certain of the stamens were also hairy.

Lasiopetala was noted in an F_3 generation and also in two cultures of an F_4 ; it is of infrequent occurrence, only 5 plants in all being observed. The plants formed persistent rosettes (*stenomeris* being annual) and only one branch produced flowers, these with hairy petals. The pollen of *lasiopetala* is 40-50 per cent. perfect; that of *stenomeris* 60-80 per cent. An F_1 progeny of 116 plants from selfed *lasiopetala* gave 60 per cent. typical *stenomeris* and 40 per cent. *lasiopetala*, thus behaving like *Oenothera lata* and *O. scintillans* in throwing their parent form *Lamarckiana*.

Of these two new types derived from *O. stenomeris* the *gigas* plant is remarkable as being another of the very few *oenotheras* discovered with the quadruploid number of chromosomes (28); triploid forms usually named *semigigas* have been described from a number of lines. The hairiness of the petals in *lasiopetala* is

² Bartlett, H. H., "The Mutations of *Oenothera stenomeris*," *Amer. Jour. Bot.*, Vol. II, p. 100, 1915.

regarded by Bartlett as a character new to the genus. Bartlett emphasizes the fact that the characters of neither type could be interpreted as the result of segregation following hybridization, which may be true, but I do not think from this that it follows that neither type can be the result of hybridization. I am not willing to admit that hybrids present only combinations of characters derived from their parent lines. It seems to me reasonable to believe that in hybrids at times the interaction of elements modifies the old or produces new factors. The species *stenomeres* and the derivatives *gigas* and *lasiopetala* have not been tested for genetic purity by cross-breeding with relatively stable types and the problems of gametic and zygotic sterility have not yet been attacked.

The final paper of this group of Bartlett's³ deals with an extremely interesting situation developed in cultures of *Oenothera Reynoldsii* from Knoxville, Tennessee. This is also a small-flowered, close-pollinated species, and its peculiarity lies in an ability to throw extraordinarily large classes of dwarfs. There are two types of dwarfs: (1) *semialta* somewhat smaller than the typical *Reynoldsii* and intermediate between it and the smaller dwarf; (2) *debilis*. A plant of *Reynoldsii* in the F₃ produced 29 individuals like itself, 32 plants of *semialta*, and 18 of *debilis*, i. e., 60 per cent. of its offspring were dwarfs. *Semialta* throws *debilis*, but no *Reynoldsii*. *Debilis* apparently can produce no *Reynoldsii* or *semialta* and breeds true except for an occasional variant *bilonga* which was also found in one culture from typical *Reynoldsii*.

With respect to the dwarfs we have here presented a beautiful series leading from the unstable parent type *Reynoldsii* through the more stable *semialta* to the most stable and most extreme dwarf *debilis*. Bartlett calls the behavior mutation *en masse*, but confesses that it bears a certain degree of resemblance to Mendelian segregation. We should very much like to see this study repeated on a larger scale and with experimental germination of the seed so that we may be sure of the ratios and also certain that the cultures have given us all of their possible progeny. Segregation *en masse* seems to the writer likely to be a more probable explanation of the phenomena than mutation.

The form *bilonga* derived from the dwarf *debilis* offers a particularly interesting problem. It is similar to *semialta* except that

³ Bartlett, H. H., "Mutation *en masse*," AMER. NAT., Vol. XLIX, p. 129, 1915.

the fruits are twice as long. The capsules sometimes reach the length of 70 mm. and average above 60 mm.; they are very much the longest fruits reported in the subgenus *Onagra*. Bartlett regards this large size of capsule as the origin of a new character. Now capsule size obviously depends upon the number of ovules produced which develop into seeds. It thus becomes an important matter to obtain the data on ovule sterility in the species *Reynoldsii* and the derived forms *semialta*, *debilis* and *bilonga*. Ovule sterility is widespread among the *Oenotheras* as all students of the genus know. Should it be found that *bilonga* produces a very much greater number of ovules than *debilis* and the other types this fact would indicate a true progressive advance. It may, however, be found that the smaller size of the capsules of *Reynoldsii semialta*, and *debilis* is due to ovule sterility, *i. e.*, to the inability of a large proportion of the ovules to set seed. This would point to a very different interpretation of the conditions in *bilonga*, and might indicate that *bilonga* is an example of reversion towards an ancestral type in which a large capsule was correlated with a high degree of ovule fertility.

In the comments which I have presented on the extremely interesting facts discovered by Bartlett no attempts have been made to offer exact explanations in line with Mendelian analysis. It is not difficult to spin hypotheses on assumptions which have been neither established nor disproved, but such creations are hardly worth the effort when the facts are within grasp. My main point is a constant questioning of the genetic purity of the material with which Bartlett has worked from the standpoint developed in my forthcoming paper "The Test of a Pure Species of *Oenothera*."⁴ It is impossible to discuss this subject in the short space of a review. The most important test is that of cross-breeding with the purest species known, to judge from the uniformity of the F_1 hybrid generation whether or not the parent types are pure. I also firmly believe that all exact genetical work on *oenotheras* must make use of methods of experimental germination to ensure complete progenies from the viable seeds and to permit the preservation of a residue of ungerminated structures that may be examined.⁵ There is in addition the determination

⁴ To appear in the *Proceedings of the American Philosophical Society*, Vol. LIV, 1915.

⁵ Davis, B. M., "A Method of Obtaining Complete Germination of Seeds in *Oenothera* and of Recording the Residue of Sterile Seed-like Structures," *Proc. Nat. Acad. Sci.*, Vol. I, p. 360, 1915.

of degrees of sterility both gametic and zygotic, and the consideration of whether such sterility is genetic or physiological. From such tests it is possible to reach much clearer conclusions on the genetic purity of *Oenothera* material than has been possible in the past.

Finally reference should be made to the important confirmation by De Vries⁶ of the studies of Stomps on *Oenothera biennis* L. In large cultures totaling 8,500 plants from Stomps's selfed line De Vries obtained 8 plants of a dwarf *biennis nanella* about 0.1 per cent., 4 plants of *biennis semigigas* (21 chromosomes) about 0.05 per cent., and 27 plants of the color variety *biennis sulfurea* about 0.3 per cent. Since the percentages from *Lamarckiana* are for *nanella* 1-2 per cent. and for *semigigas* 3 per cent., it would appear that *biennis* is the more stable of the two species, although the color variety *biennis sulfurea* is a new type in experimental studies in *Oenothera*. A culture of over 1,000 plants from selfed seed of *biennis sulfurea*, all with pale yellow flowers, produced 2 dwarfs, thus giving what De Vries calls a "double mutant," *O. biennis* mut. *sulfurea* mut. *nanella*.

This behavior of *Oenothera biennis* is to the writer much more trustworthy evidence for mutation than that presented from the studies on *Lamarckiana* since *biennis* has a record of a long history as a species on the sand hills of Holland, where there appears to have been little probability of recent contamination. However, the showing of "mutants" from *biennis* does not appear very encouraging for the mutation theory of organic evolution when it is remembered that *biennis nanella* is frequently weakly or diseased, that *biennis semigigas* is self sterile, and that *biennis sulfurea* appears to be a retrogressive form having lost the power of producing normal yellow flowers. Although the Dutch *biennis* of all the *oenotheras* so far brought into the experimental garden still seems to me the form most free from suspicion of genetic impurity, nevertheless, the line of Stomps's has not, so far as we know, been subjected to all of the tests of a pure species. Until these tests are made it is not safe to assume that this material is wholly pure. It seems to me not improbable that other species of *Oenothera* will eventually be isolated more stable than the Dutch *biennis*.

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June, 1915

⁶ De Vries, Hugo, "The Coefficient of Mutation in *Oenothera biennis* L.," *Bot. Gaz.*, Vol. LIX, p. 169, 1915.

THE VALUE OF INTER-ANNUAL CORRELATIONS

If $x_1, x_2, x_3 \dots x_n$ be measures taken on the n individuals of a series in a given year and $x'_1, x'_2, x'_3 \dots x'_n$ be similar measures taken in a subsequent year, the correlation between the first and second measures on the same individual $r_{xx'}$, may be designated as a direct inter-annual correlation.¹ The purpose of this review is to illustrate the usefulness of such constants, with a view to extending their application, by bringing together examples of inter-annual correlations from various fields.

The immediate value of such coefficients may be purely scientific, economic, or both theoretical and practical.

Practically such means of prediction as correlation and regression formulæ should find wide application in breeding operations where it is desirable to weed out or send to the butcher at the earliest possible moment those individuals which can not be kept with the maximum profit. If the correlation between the egg production of a fowl in her pullet year and her laying capacity in any subsequent year be high, it is clear that those which on the average are to prove unprofitable may be sent to the pot when most desirable for that purpose, and before they have consumed two or more years' feed without yielding the maximum return in eggs. If, on the contrary, there be no correlation, the labor of selection in the pullet year is an unnecessary expense. If a cow's milking capacity be closely correlated with her milking record in her heifer year, the culling of dairy herds may be profitably carried out in the first year. In plant breeding experiments, involving either sexual or vegetative reproduction, selection of individuals for future propagation must be made, and at as early a date as possible. If the future yield per plant of hay can be estimated with considerable accuracy from a first year's culture the process of selecting clonal strains can be carried out with far greater rapidity than if one must wait for the results of subsequent years' tests. In all such cases the finality of a first judgment must depend in large degree upon the closeness of correlation between the results of successive experiments—in short upon the value of the inter-annual correlation coefficient.

¹ Cross inter-annual correlations in which the measures taken are of a different sort are sometimes useful, but examples of such are not considered in this review.

In dealing with egg production Pearl and Surface² give as the correlation in number of eggs for first and second year

$$r = .032 \pm .083,$$

a value which, though positive, is clearly insignificant with regard to its probable error.

Thus in this particular case the performance of the first year furnishes no clue to that of the second. With respect to egg-laying capacity, the record of the pullet year furnishes no criterion for elimination from the flock.

For milk yield in cattle the case seems to be quite different. Gavin³ has found that there is a medium correlation between (a) the "revised maximum"⁴ yield in quarts of successive lactations, and (b) between the "revised maximum" of the individual lactation periods and the highest revised maximum reached by the animal.

So far as I am aware the only worker who has published correlations between the characters of the same plant individuals in different years is Clark⁵ whose results have been noted in these pages by Pearl.⁶

The correlation tables and constants show that plants of a given class in any year (height or weight of hay produced) will be highly variable in a subsequent year, but will on the average deviate from the mean of the whole culture of the year in the same direction and to about half the extent of the type selected in the preceding year. Thus if selection were made on the basis of a single year's test only, many individual plants of low yield would be discarded which in a subsequent year would have taken higher rank, while high-yielding plants would be retained which subsequently would give disappointing results. On the whole, however, the yield of a hay plant one year does furnish a valuable index to its yield in a subsequent year.

² Pearl, R. and F. M. Surface, "A Biometrical Study of Egg Production in the Domestic Fowl," I, *Bull. Bu. Anim. Ind.*, 110, 66, 1909.

³ Gavin, Wm., "Studies in Milk Records: On the Accuracy of Estimating a Cow's Milking Capacity by Her First Lactation Period." *Jour. Agr. Sci.*, 5, 377-390, 1913.

⁴ "Revised Maximum" milk yield is the maximum day yield which is three times reached or exceeded in a lactation.

⁵ Clark, C. F., "Variation and Correlation in Timothy," *Bull. Cornell Agr. Exp. Sta.*, 279, 1910.

⁶ Pearl, R., *AMER. NAT.*, 45, 418-419, 1911.

That we are dealing with a real measure of the relatively permanent differentiation of individuals, and not with merely temporary differences due to growth, is indicated by the fact that the correlations between a first and a third year are about the same as those between a first and a second or a second and a third.

In other fields of plant industry such methods may be profitably applied. For example Sievers⁷ after discussing at some length the question of the differentiation of belladonna plants with respect to alkaloidal content, warns the reader that "the investigation has hardly progressed far enough to yield any definite conclusions" but says in summarizing his data:

A considerable number of plants with leaves rich in alkaloids in one season are found to have equally rich leaves in the following season. Furthermore, they frequently manifest the same characteristics at the various stages of growth during the season in comparison with other plants. The same facts are true with regard to plants which bear leaves with a low percentage of alkaloids.

How much more definite is the information conveyed by the simple statement that the inter-annual correlation⁸ between the alkaloidal content for 1911 and 1912 is

$$r = .513 \pm .066!$$

Such studies as those by Stockberger on individual performance in hops⁹ may be facilitated by the use of inter-annual correlation coefficients. He gives only the extremes of his series of individuals, but from these the correlations between yield per hill for different years are:

	Lowest Hills	Highest Hills
1909 and 191029 \pm .17	.59 \pm .13
1910 and 191155 \pm .13	.52 \pm .14
1909 and 191143 \pm .15	.30 \pm .18

Such constants, deduced from materials which almost certainly

⁷ Sievers, A. F., "Individual Variation in the Alkaloidal Content of Belladonna Plants," *Jour. Agr. Res.*, 1, 129-146, 1913.

⁸ In computing this coefficient a number of inconsistencies in the data table were discovered. The constant as given is probably as nearly correct as can be found from the available data.

⁹ Stockberger, W. W., "A Study of Individual Performance in Hops," *Prac. Amer. Breed. Ass.*, 7, 452-457, 1912.

do not show the full strength of the correlation, remove at once all question concerning the relatively permanent differences in productiveness of the individual hills.

Consider next an illustration from hybridization of measurable characters.

Goodspeed and Clauson¹⁰ have given the mean values of measurements of the flowers of individual plants of *Nicotiana* hybrids cultivated in 1912 and of corollas of the same plants cut back and flowered in 1913. The correlations between the mean dimensions for the two years I find to be:

N. Tabacum var. *macrophylla* ♀ × *N. sylvestris* ♂

F₁ plants, N=21.

For spread of corolla, $r = .044 \pm .147$.

For length of corolla, $r = .169 \pm .143$.

Hybrid produced by crossing F₁ of the hybrid *N. Tabacum* "Maryland" ♀ by *N. Tabacum* ♂, with *N. sylvestris*, N=19.

For spread of corolla, $r = .560 \pm .106$.

For length of corolla, $r = .788 \pm .059$.

These correlations show at once the high degree of uniformity of the F₁ of the first as compared with that of the second series. In all four cases the signs of the coefficients are positive, but those of the first class are insignificant in comparison with their probable errors. In both cases length of corolla is more closely correlated than breadth. Possibly this is due to errors of sampling only, or to greater difficulty in obtaining an exact measure of the spread of the limb. It may, however, indicate that some characters are more sharply and permanently differentiated from individual to individual than others.

That the latter may sometimes be the case is clearly shown by unpublished data of my own for the ligneous perennials *Staphylea trifolia* and *Hibiscus Syriacus*.¹¹

¹⁰ Goodspeed, J. H., and R. E. Clauson, "Factors Influencing Flower Size in *Nicotiana* with Special Reference to Questions of Inheritance," *Amer. Jour. Bot.*, 2, 232-274, 1915.

¹¹ The constants are based in all cases on mean values of the characters of ovaries of shrubs well established in the Missouri Botanical Garden. In such work the number of individuals can never for practical reasons be very large, if a fairly large number of countings be made for each shrub. Furthermore much of the work which one does may be lost by some accident which precludes the securing of countings from each individual every year. If an individual is not represented in both of a pair of years it must be omitted entirely.

The accompanying tables show the correlations deduced for the characters indicated.¹²

INTER-ANNUAL CORRELATIONS FOR FRUITS OF STAPHYLEA

Relationship	Correlation for 1906 and 1908, n = 19	Correlation for 1907 and 1908, n = 15	Correlation for 1908 and 1909, n = 20
Ovules and ovules.....	.445 ± .069	.816 ± .058	.872 ± .036
Seeds and seeds.....	.063 ± .154	.064 ± .173	.056 ± .150
Asymmetry and asymmetry.....	.748 ± .068	.102 ± .172	.205 ± .145
Locular composition and locular composition ..	.601 ± .099	.294 ± .159	.335 ± .134

INTER-ANNUAL CORRELATION FOR FRUITS OF HIBISCUS, n = 23.

Relationship	Correlation for 1907 and 1908
Sepals and sepals451 ± .112
Bracts and bracts836 ± .042
Ovules and ovules941 ± .016
Seeds and seeds630 ± .085
Asymmetry and asymmetry747 ± .062
Locular composition and locular composition725 ± .067
Fertility and fertility610 ± .088
Correlation and correlation035 ± .141

The constants are very irregular in magnitude, but are without exception positive in sign. In many instances they are large. Thus in these individual shrubs which taxonomically show no differences¹³ there is nevertheless a distinct differentiation in respect of the great majority of the characters examined.

While the probable errors are large the evidence warrants the conclusion that some are decidedly more highly correlated than others.

¹² Sepals = mean number of sepals in calyx.

Bracts = mean number of bracts in involucre.

Ovules = mean number of ovules formed per fruit.

Seeds = mean number of seeds matured per fruit.

Asymmetry = average radial asymmetry in the distribution of the number of ovules per locule. For method of computation see *Biometrika*, Vol. VII, pp. 477-478, 1910, and *AMER. NAT.*, Vol. XLVI, p. 480, 1912.

Locular composition = average number of locules per fruit with an odd number of ovules. See citations above.

Fertility = coefficient of fertility (mean seeds per fruit) (mean ovules per fruit).

Correlation = coefficient of correlation between number of ovules and number of seeds per locule.

¹³ I believe one of the *Hibiscus* shrubs had lighter flowers than the rest.

In *Hibiscus* the differentiation of the individuals with respect to number of bracts seems to be greater than that for number of sepals. For both *Staphylea* and *Hibiscus* the correlation for ovules is generally high. It is in every instance higher than that for mean number of seeds matured per fruit. Correlation for both mean number of seeds per fruit and relative number of seeds matured has a moderately large value in *Hibiscus*, but in *Staphylea* it is sensibly 0. In both species such characteristics of the ovary as radial asymmetry and locular composition seem to be rather sharply differentiated from individual to individual. This is probably due in part to differentiation with respect of number of ovules per fruit, but further discussion of the problem would be out of place in a note, the only purpose of which is to call attention to the usefulness, in both applied and pure science, of a quantitative means of detecting and expressing permanent differentiation.

In this brief review I have made no attempt to discuss fully all the biological phases of the problems suggested. The analysis of the data may in several instances be carried much further by the use of the statistical tools. Perhaps enough has been said to indicate that inter-annual coefficients may be of real service in practical animal husbandry, in plant breeding and in morphology and physiology. More than usefulness is not to be expected of any method.

J. ARTHUR HARRIS

THE PHENOMENON OF SELF STERILITY

In my paper which appeared in THE AMERICAN NATURALIST, Vol. XLIX, p. 79, the last seven lines on page seventy-nine should read as follows:

Self-sterile plants crossed with self-sterile plants gave only self-sterile offspring. Certain self-fertile plants, however, gave only self-fertile offspring either when self-pollinated or when crossed with self-sterile plants. Other self-fertile plants gave ratios of 3 self-fertile to 1 self-sterile offspring when self-pollinated, and ratios of 1:1 when crossed with pollen from self-sterile, etc.

E. M. EAST.

